Infectious Mononucleosis and Risk for Multiple Sclerosis: A Meta-analysis

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Objective: **To characterize the association between infectious mononucleosis (IM), a frequent clinical manifestation of primary Epstein–Barr virus infection after childhood, and the risk for multiple sclerosis (MS).** ***Methods:*** **We conducted a systematic review and meta-analysis of case–control and cohort studies of IM and MS.** ***Results:*** **The combined relative risk of MS after IM from 14 studies was 2.3 (95% confidence interval, 1.7–3.0; $p < 10^{-8}$). Potential sources of heterogeneity (ie, study design, MS definition, and latitude) barely influenced our results.** ***Interpretation:*** **We conclude that Epstein–Barr virus infection manifesting as IM in adolescents and young adults is a risk factor for MS.**

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Viral infections have long been suspected of playing a role in multiple sclerosis (MS).[1] Increasing evidence implicates infection with Epstein–Barr virus (EBV) as an important MS risk factor.[2] The similarities in the epidemiology of infectious mononucleosis (IM) and MS led investigators to consider EBV in the cause of MS some 25 years ago.[1] Both IM and MS occur in young adults, both follow a latitude gradient, and both are rare in populations where infections occur at an early age, suggesting that late infection with EBV, evidenced by occurrence of IM, is an important causal factor in MS.[1,3]

However, studies that have evaluated the relation of IM and MS risk have not produced consistent results. A number of case–control studies and a well-designed cohort study found an increased risk for MS in individuals with a history of IM,[3,5–9] whereas several case–control studies and two cohort studies did not find any difference.[4,10–16] In this review, we systematically identify and combine the relevant studies of the association between IM history and MS to produce a summary measure of the association.

Subjects and Methods

Search Strategy

We searched Medline without language restrictions from 1965 through March 2005 using the following terms: Epstein–Barr virus *or* EBV *or* human herpesvirus 4 *or* HHV-4 *or* infectious mononucleosis *or* glandular fever, *and* multiple sclerosis *or* MS *or* disseminated sclerosis. We also reviewed bibliographies, searched the Science Citation Index Expanded database, contacted authors and experts, and searched Medline for all case–control studies of MS to identify reports including IM only as a minor topic.

Data Extraction

Publications reporting results from analytic epidemiological studies of IM and MS were eligible for extraction. Two of us independently extracted relative risks (RRs), confidence intervals (CIs), and other information from each study, resolving discrepancies by joint review. We assigned latitude for each study as the latitude of the nearest major city to where the study was conducted or where the majority of study subjects lived.

Statistical Analyses

We based our meta-analysis on DerSimonian and Laird's[17] random-effects model implemented in Stata version 9.0 (Stata Corporation, Stanford, CA).[18] According to a priori criteria jointly evaluated by two of us, studies had to have crude or adjusted RR and CI reported in the article, estimable from data provided in the article or from additional information obtained by personal contact with authors to be included in the meta-analysis. After completing the search, however, we noticed that two studies reported a lack of significant association between IM and MS without providing a RR estimate or CI. Because exclusion of these studies could bias the summary RR, we included these studies assuming a RR of 1.0 and estimating CI based on the number of reported MS cases and the likely population prevalence of IM. For studies reporting adjusted results, the adjusted results were entered into the meta-analysis to minimize the effects of confounding. For studies with only crude results, the crude

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results were entered into the meta-analysis. Studies were weighted by the precision of the RR, based on the 95% CI.

To check the robustness of our meta-analysis, we repeated the analysis under various assumptions of precision in studies for which we estimated CIs without complete data, and we assessed the influence of individual studies on the combined RR by dropping each study one at a time. Furthermore, we used stratified analyses and meta-analysis regression to assess the effects of several variables on heterogeneity among the individual study's RRs, including study design (case–control vs cohort), MS case definition (ie, definite vs definite + probable; incident vs prevalent), reported association (crude vs adjusted), and latitude of study locations. A funnel plot and a regression asymmetry plot were used to check for publication bias.[19,20] p less than 0.05 was considered significant.

Results

Our search found 295 publications. We excluded 280 studies; common exclusions were reports on biochemistry, virology, and immunology and studies of risk factors for MS other than IM. Of the 15 studies retained for full extraction, we excluded 1 study[21] because it contained insufficient data to calculate the RR and we received no reply from our attempt to retrieve the data from the authors. The remaining 14 studies[3–16] are summarized in Table 1. Two of these[12,13] reported null results without specifying the RR and CI. Although we contacted the authors of these articles, neither could provide us with the necessary data, so we assumed RR = 1 and calculated the CI as described earlier.

The 14 studies in the meta-analysis included 11 case–control studies[3,5–8,11–16] and three cohort studies.[4,9,10] RRs, reported as odds ratios or risk ratios, ranged from 0.8 to 17, with a median of 2.5. Meta-analysis (Fig 1) produced a combined RR of 2.3 (95% CI, 1.7–3.0; $p < 10^{-8}$). There was no significant heterogeneity among the 14 individual study results ($Q_{df=13} = 16.074$; $p = 0.245$).

Stratified meta-analyses showed a slightly higher combined RR for cohort studies versus case–control studies, studies of incident MS cases versus prevalent cases, and studies limited to definite MS cases versus definite and probable cases, but none of these differences was statistically significant. Latitude of study locations was not sufficiently varied to detect heterogeneity due to latitude. Six studies reporting only crude results had significantly lower RRs than studies report-

Table 1. Studies of the Association between Infectious Mononucleosis and Multiple Sclerosis

Author, Year, Country	Cases (N): Control Subjects (N)	Female: Male Cases Ratio	Case Inclusion Criteria	Case Source	Control or Comparison Cohort Source	Exposure Ascertainment	RR (95% CI)
Case–control studies							
Operskalski,[5] 1989, United States	145:145	4.4:1	Definite/probable MS	MS cohort (prevalent)	Friends of cases	Interview or questionnaire	17.0 (2.0–81.8)
Souberbielle,[11] 1990, France	153:153	1.7:1	Definite MS Poser criteria	Hospital (incident)	Hospital	Interview	1 (0.19–5.37)
Hopkins,[12] 1991, United States	16:61	4.3:1	Definite/probable MS Poser criteria	Community (prevalent)	Community	Interview	1 (0.09–5.55)[a]
Martyn,[6] 1993, United Kingdom	214:160	2.12:1	1. Definite MS 2. Optic neuritis 3. Isolated demyelination	Hospital (prevalent)	1. Hospital 2. Blood donors	Interview	2.9 (1.1–7.2)
Casetta,[13] 1994, Italy	104:150	2.1:1	Definite MS McAlpine criteria	Community (prevalent)	1. Hospital 2. Community	Interview	1 (0.31–3.06)[a]
Gusev,[14] 1996, Russia	155:155	1.63:1	Definite/probable MS McAlpine criteria	Community (incident and prevalent)	1. Hospital 2. Hospital staff	Interview	3.03 (0.24–160.55)
Marrie,[7] 2000, United Kingdom	225:900	2.3:1	Definite/probable MS	Community (incident)	Community	Medical record	5.5 (1.5–19.7)
Hernan,[3] 2001, United States	301:1416	All female	Definite/probable MS Poser criteria	Cohort study (incident and prevalent)	Cohort study	Questionnaire	2.2 (1.6–3.0)
Zorzon,[15] 2003, Italy	140:131	1.8:1	MS McDonald criteria	MS center (prevalent)	Blood donors	Interview	0.8 (0.3–2.2)
Haahr,[16] 2004, Denmark	53:53	12.3:1	Neurologist diagnosed MS	MS Society (Incident)	Friends of cases	Questionnaire	3.58 (0.97–16.23)
Ponsonby,[8] 2005, Australia	136:272	2.1:1	Definite MS Poser criteria and imaging	1. MS Society 2. Neurologists (prevalent)	Voter rolls	Interview or questionnaire	2.01 (1.11–3.62)
Cohort studies							
Lindberg,[10] 1991, Sweden	3 cases	Not reported	Definite MS Poser criteria or laboratory results	Hospital (incident)	Regional population	Medical record	3.7 (0.8–17.6)
Haahr,[9] 1995, Denmark	16 cases	1.6:1	Definite/probable MS Allison criteria and laboratory results	MS registry (incident)	National population	Heterophile antibody test	2.8 (1.6–4.6)
Goldacre,[4] 2004, United Kingdom	6 cases	Not reported	Hospital admission for MS	Hospital records (incident)	Hospital records	Medical record	2.17 (0.79–4.77)

[a]Confidence intervals for these studies could not be calculated from data given in the articles or furnished by authors, and were calculated based on the number of reported MS cases and the likely population prevalence of infectious mononucleosis.

RR = relative risk; CI = confidence interval; MS = multiple sclerosis.

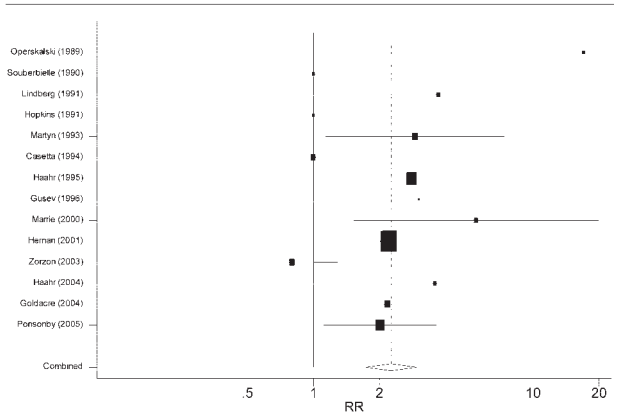

Fig 1. Random-effects meta-analysis of 14 studies of the association between infectious mononucleosis and multiple sclerosis. Confidence intervals are truncated at 20. RR = relative risk.

ing adjusted results ($p = 0.01$). However, most of these crude studies were imprecise, contributing little weight to the meta-analysis. Studies reporting adjusted RRs, taken as a subgroup, produced a combined RR of 2.5 (95% CI, 2.0–3.2; $p < 10^{-12}$), with no significant heterogeneity ($Q_{df=7} = 7.389$; $p = 0.390$), which is similar to the main meta-analysis. Results from the stratified analyses and meta-analysis regression are given in Table 2.

We experimented with various precision levels for the two studies reporting null results, for which we calculated CIs.[12,13] Even assuming tight precision (RR = 1.0; 95% CI, 0.9–1.1), the combined RR remained significantly above the null, the combined CI covering the point estimate of 2.3 from the primary meta-analysis. This strongly suggests that the true association is indeed nonnull, likely close to 2.3. We identified one unpublished study of IM and MS (by Dr B.M. Zaadstra), reporting a RR of 2.1 and 95% CI of 1.7 to 2.8. Because we were unable to obtain details about this study, we did not include it in our main analysis. Adding these data barely changed our result: combined RR = 2.2 (95% CI, 1.8–2.7; $p < 10^{-14}$), with no significant heterogeneity ($Q_{df=14} = 16.249$; $p = 0.298$).

Excluding studies from the main analysis one at a time demonstrated that no single study was overly influential. The average combined RR excluding single studies was virtually identical to the combined RR from the main meta-analysis. Tests and plots for publication bias gave null results.

Discussion

The results of this study combined with those of serological investigations[22–24] suggest that the risk for MS, which is extremely low in individuals who are not infected with EBV, increases after infection. Replication of the positive association between IM and MS in many studies suggests that the association is not a product of chance. It is possible that the positive association in case–control studies arose spuriously through recall bias, as many of these studies ascertained the occurrence of IM retrospectively through interview or questionnaire, but the consistency of results between case–control and cohort studies suggests that the influence of this source of error was at most modest. A spurious positive association between IM and MS could also have occurred if individuals with history of IM were more likely to be diagnosed with MS than individuals without such history. This appears to be an unlikely proposition, because in the majority of studies in the meta-analysis,[3,8–15] the diagnosis of MS was based

Table 2. Stratified Meta-analysis and Meta-analysis Regression for Assessing Potential Sources of Heterogeneity among Studies of the Association between Infectious Mononucleosis and Multiple Sclerosis

Variable	Studies, n	RR (95% CI)	p for Meta-analysis Regression Coefficient
Study design			0.30
Case–control studies[3,5–8,11–16]	11	2.1 (1.5–3.1)	
Cohort studies[4,9,10]	3	2.7 (1.7–4.2)	
MS occurrence			0.11
Incident[7,9–11,16]	5	3.0 (2.0–4.6)	
Incident + prevalent[3,4,14]	3	2.2 (1.6–3.0)	
Prevalent[5,6,8,12,13,15]	6	1.9 (1.0–3.6)	
MS diagnosis certainty[a]			0.27
Definite[8,10,11,13]	4	1.8 (1.1–2.9)	
Definite + probable[3,5–7,9,12,14]	7	2.7 (1.9–3.8)	
Reported association			0.01
Crude[11–16]	6	1.2 (0.7–2.1)	
Adjusted[3–10]	8	2.5 (2.0–3.1)	
Latitude[3–16]	14	NA	0.33

[a]Three studies[4,15,16] did not report the MS diagnosis certainty.

RR = relative risk; CI = confidence interval; MS = multiple sclerosis; NA = not applicable.

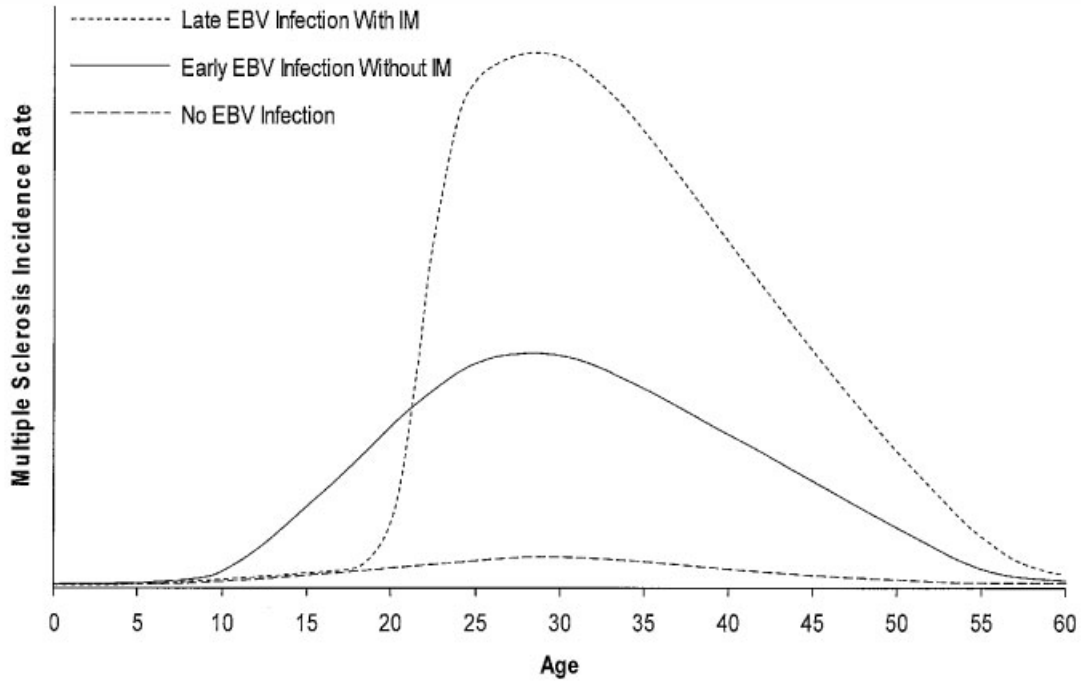

Fig 2. Schematic representation of multiple sclerosis (MS) incidence according to Epstein–Barr virus (EBV) infection. The shape of the incidence curve labeled "Early EBV infection without IM" is based on the typical age-specific incidence of MS in most populations; incidence begins to increase in adolescence, peaks around age 25 to 30 years, and declines to nearly zero by age 60.[25] The age-specific incidence for the group with no EBV infection has been drawn at one-tenth the incidence among EBV-positive individuals, based on the results of a previous review,[26] and that of individuals with late EBV infection and infectious mononucleosis (IM) has been estimated to be 2.3 times higher than that of EBV-positive individuals without history of IM (this meta-analysis). More accurate curves could be drawn by taking into account the proportion of individuals in the population who are infected with EBV early in childhood and the age-specific prevalence of history of IM. We have ignored these adjustments for simplicity, and because these proportions vary across developed countries.[27]

on standard criteria, minimizing the possibility that knowledge of exposure would influence diagnosis.

Using the results of this meta-analysis and results from other investigations, we can conceptualize a model of the relation between EBV infection and MS that accounts for early-life infection versus infection in adolescence or adulthood, which often manifests as IM[24] (Fig 2). According to this model, the risk for MS is close to zero among individuals who are not EBV infected, intermediate among individuals infected with EBV in early childhood, and highest among individuals first infected with EBV in adolescence or later in life. This model predicts that many cases of MS could be prevented by a suitable vaccine that protects against EBV infection. A smaller but still substantial number of cases could be prevented by exposing children to EBV infection early in life. A critical test of the proposed model is difficult in the absence of a safe and effective vaccine, but corroborating evidence could be accrued by conducting large longitudinal studies of EBV infection and MS risk, by investigating the genetic and environmental factors (including other infections) that interact with EBV, and by elucidating the molecular mechanism that may link EBV or the immune response to EBV to MS. These studies could be extended to other autoimmune diseases, particularly to systemic lupus erythematosus, which also is strongly associated with EBV infection.[28] An early event in systemic lupus erythematosus appears to be the appearance of antibodies to EBV that cross-react with a lupus autoantigen.[29] Molecular mimicry mechanisms have also been proposed for MS,[30,31] but their causative relevance remains to be proved. Whereas there is no doubt that EBV is at best only one component in the complex pathway of events that cause MS, it could prove to be one of the components that is more vulnerable to intervention.

References

1. Ascherio A, Munger K. Multiple sclerosis. In Nelson LM, ed. Neuroepidemiology: from principles to practice. Oxford: Oxford University Press, 2004.
2. Ascherio A, Munch M. Epstein-Barr virus and multiple sclerosis. Epidemiology 2000;11:220–224.
3. Hernan MA, Zhang SM, Lipworth L, et al. Multiple sclerosis and age at infection with common viruses. Epidemiology 2001; 12:301–306.

4. Goldacre MJ, Wotton CJ, Seagroatt V, Yeates D. Multiple sclerosis after infectious mononucleosis: record linkage study. J Epidemiol Community Health 2004;58:1032–1035.
5. Operskalski EA, Visscher BR, Malmgren RM, Detels R. A case-control study of multiple sclerosis. Neurology 1989;39: 825–829.
6. Martyn CN, Cruddas M, Compston DA. Symptomatic Epstein-Barr virus infection and multiple sclerosis. J Neurol Neurosurg Psychiatry 1993;56:167–168.
7. Marrie RA, Wolfson C, Sturkenboom MC, et al. Multiple sclerosis and antecedent infections: a case-control study. Neurology 2000;54:2307–2310.
8. Ponsonby AL, van der Mei I, Dwyer T, et al. Exposure to infant siblings during early life and risk of multiple sclerosis. JAMA 2005;293:463–469.
9. Haahr S, Koch-Henriksen N, Moller-Larsen A, et al. Increased risk of multiple sclerosis after late Epstein-Barr virus infection: a historical prospective study. Mult Scler 1995;1:73–77.
10. Lindberg C, Andersen O, Vahlne A, et al. Epidemiological investigation of the association between infectious mononucleosis and multiple sclerosis. Neuroepidemiology 1991;10:62–65.
11. Souberbielle BE, Martin-Mondiere C, O'Brien ME, et al. A case-control epidemiological study of MS in the Paris area with particular reference to past disease history and profession. Acta Neurol Scand 1990;82:303–310.
12. Hopkins RS, Indian RW, Pinnow E, Conomy J. Multiple sclerosis in Galion, Ohio: prevalence and results of a case-control study. Neuroepidemiology 1991;10:192–199.
13. Casetta I, Granieri E, Malagu S, et al. Environmental risk factors and multiple sclerosis: a community-based, case-control study in the province of Ferrara, Italy. Neuroepidemiology 1994;13:120–128.
14. Gusev E, Boiko A, Lauer K, et al. Environmental risk factors in MS: a case-control study in Moscow. Acta Neurol Scand 1996; 94:386–394.
15. Zorzon M, Zivadinov R, Nasuelli D, et al. Risk factors of multiple sclerosis: a case-control study. Neurol Sci 2003;24: 242–247.
16. Haahr S, Plesner AM, Vestergaard BF, Hollsberg P. A role of late Epstein-Barr virus infection in multiple sclerosis. Acta Neurol Scand 2004;109:270–275.
17. DerSimonian R, Laird NM. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–188.
18. Sharp S, Sterne J. sbe16: meta-analysis. Stata Tech Bull 1997; 38:9–14.
19. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50: 1088–1101.
20. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315:629–634.
21. Bachmann S, Kesselring J. Multiple sclerosis and infectious childhood diseases. Neuroepidemiology 1998;17:154–160.
22. Ascherio A, Munger KL, Lennette ET, et al. Epstein-Barr virus antibodies and risk of multiple sclerosis. JAMA 2001;286: 3083–3088.
23. Alotaibi S, Kennedy J, Tellier R, et al. Epstein-Barr virus in pediatric multiple sclerosis. JAMA 2004;291:1875–1879.
24. Levin LI, Munger KL, Rubertone MV, et al. Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA 2005;293:2496–2500.
25. Koch-Henriksen N. The Danish Multiple Sclerosis Registry: a 50-year follow-up. Mult Scler 1999;5:293–296.
26. Ascherio A, Munch M. Epstein-Barr virus and multiple sclerosis. Epidemiology 2000;11:220–224.
27. Niederman JC, Evans AS. Epstein-Barr virus. In: Evans AS, Kaslow RA, eds. Viral infections of humans: epidemiology and control. New York: Plenum Publishing, 1997.
28. James JA, Kaufman KM, Farris AD, et al. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. J Clin Invest 1997;100:3019–3026.
29. McClain MT, Heinlen LD, Dennis GJ, et al. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. Nat Med 2005;11:85–89.
30. Lang HL, Jacobsen H, Ikemizu S, et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol 2002;3:940–943.
31. Rand KH, Houck H, Denslow ND, et al. Epstein-Barr virus nuclear antigen-1 (EBNA-1) associated oligoclonal bands in patients with multiple sclerosis. J Neurol Sci 2000;173:32–39.