

Multiple sclerosis and Epstein-Barr virus

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OBJECTIVE: To evaluate the epidemiological evidence for an etiological role of Epstein-Barr virus in multiple sclerosis (MS).

DATA SOURCES: MEDLINE and Cochrane Library searches of the medical literature identified 24 studies.

DATA EXTRACTION: Studies were categorized as seroepidemiological, case-control or historical cohort, and were then classified within each group according to methodological rigour using criteria derived from published guidelines for the epidemiological study of MS.

DATA SYNTHESIS: There was significant variability in the quality of evidence, and while two well-designed cohort studies found increased relative risks of MS in subjects with infectious mononucleosis, results from other studies were unconvincing.

CONCLUSIONS: The evidence was insufficient to accept or reject the hypothesis that Epstein-Barr virus has an etiological role in MS. Further study, ideally using large samples of incident cases with blinded, trained interviewers using confirmatory sources for recalled data, is needed.

Key Words: *Epstein-Barr virus; Infectious mononucleosis; Multiple sclerosis; Review*

Sclérose en plaques et virus Epstein-Barr

OBJECTIF : Évaluer les données épidémiologiques quant au rôle étiologique du virus Epstein-Barr dans la sclérose en plaques (SP).

SOURCES DE DONNÉES : Les recherches dans MEDLINE et la bibliothèque Cochrane ont permis de relever 24 études.

EXTRACTION DES DONNÉES : Les études ont d'abord été divisées en trois catégories : séro-épidémiologiques, cas/témoins et cohortes antérieures, puis classées, dans chacun des groupes, en fonction de leur rigueur méthodologique selon des critères définis dans des lignes directrices publiées concernant l'étude épidémiologique de la SP.

SYNTHÈSE DES DONNÉES : Des écarts importants ont été notés relativement à la qualité des données; deux études de cohortes, bien conçues, ont fait ressortir des risques relatifs accrus de SP chez les sujets ayant subi une mononucléose infectieuse, tandis que les résultats des autres études n'étaient pas concluants.

CONCLUSION : Nous ne disposons pas suffisamment de données pour confirmer ou infirmer l'hypothèse selon laquelle le virus Epstein-Barr joue un rôle étiologique dans la SP. Il faudrait donc poursuivre l'étude, idéalement à partir de grands échantillons de cas nouveaux et en faisant appel à des intervieweurs formés et tenus dans l'ignorance, qui utilisent des sources de confirmation pour le rappel des données.

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS) of unknown etiology. Recently, a multifactorial etiology was proposed, in which multiple environmental factors act together in a genetically susceptible individual to cause the disease (1). Geographic and temporal variation in incidence and prevalence, as well as an apparently age-depend-

ent change in disease risk with migration, support an etiological role for environmental factors (1).

Viral infection is touted as a putative etiological factor in MS. Animal models of virally mediated CNS demyelination exist, although the mechanisms are unknown (2). Viruses of the herpesvirus family are of interest because of their neurotropism, ubiquitous nature and tendency to pro-

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TABLE 1
Seroepidemiological studies – Characteristics

Author (reference)	Diagnostic criteria	Cases		Source	Comparison Group			Rating
		Number	Exclusions		Source	Number	Matching	
Myhr et al, 1998 (19)	McAlpine et al (46) – CDMS, CPMS, CPoMS	144 prevalent	None	Hordaland County, Norway; onset 1976 to 1986, diagnosed before January 1, 1987	Hospital patients with traumatic fractures, ligament rupture, and minor gynecological or plastic surgeries	170	Age (five- year groups), sex, residence	B
Munch et al, 1998 (18)	Poser et al (36)	8 prevalent	None	Fjelso, Denmark MS patients who attended school or scouts together	Healthy schoolmates; random healthy persons; MS patients from elsewhere in Denmark; patients with auto- immune disease	44	None	B
Shirodaria et al, 1987 (21)	Allison and Millar (47) – CPMS	26 prevalent	NR	NR	Rheumatoid arthritis patients; healthy blood donors	52	Age ± two years, sex	B
Larsen et al, 1985 (17)	CDMS, LSDMS	100 prevalent	NR	NR	Healthy hospital staff; blood donors	100	Age, sex	B
Sumaya et al, 1985 (24)	Schumacher et al (48), Rose et al (25) – CDMS	104 prevalent	NR	NR	Healthy non-blood- related subjects (matched); healthy siblings; adults with other neurological diseases	175	Age ± three years, sex	B
Bray et al, 1983 (11)	Two neurologists – CDMS, LSDMS	313 prevalent	None	Consecutive patients	Normal blood donors; patients with nondemyelinating neurological diseases	406	Age, sex	B
Sumaya et al, 1980 (23)	Schumacher et al (48) – CDMS	157 prevalent	NR	University of California at Los Angeles (Los Angeles, USA) MS Clinic	Spouses; non-blood- related household members; laboratory personnel	81	None	B
Enbom et al, 1997 (15)	Schumacher et al (48) – CDMS, optic neuritis	55 prevalent	Other optic nerve or neurological diseases	NR	Other neurological diseases	40	NR	B
Bray et al, 1992 (12)	Schumacher et al (48), Poser et al (36) – CDMS, intact blood-brain barrier	50 prevalent	NR	NR	Other neurological diseases with intact blood-brain barrier needing lumbar punc- ture; positive Epstein- Barr virus serology	50	NR	C
Compston et al, 1986 (13)	Acute relapsing CDMS, optic neuritis, isolated demyelinating lesion	177 incident, prevalent	>50 years old	Patients at National Hospitals for Nervous Disease, QueenSquare or Maida Vale, Moorfields Eye Hospital (London, England)	Patients with noninfectious or immune-mediated neurological disorders; blood donors	164	None	C
Nikoskelainen, et al, 1972 (20)	NR	52 prevalent	NR	NR	Siblings, controls	91	Age, sex, residence	C
Ellison et al, 1977 (14)	NR	93 prevalent	NR	NR	Normal controls	54	NR	C
Kinnunen et al, 1990 (16)	NR	19 prevalent	NR	Finnish Twin Cohort	Unaffected twin	19	Not applicable	C

CDMS Clinically definite multiple sclerosis; CPMS Clinically probable multiple sclerosis; CPoMS Clinically possible multiple sclerosis; LSDMS Laboratory-supported definite multiple sclerosis; LSPMS Laboratory-supported probable multiple sclerosis; MS Multiple Sclerosis; NR Not reported

TABLE 2
Seroepidemiological studies – Methods and results

Author (reference)	Exposure ascertainment		Results	Rating
	Blinded*	Methods		
Myhr et al, 1998 (19)	NR	Serology	Cases: higher seropositive rate [†]	B
Munch et al, 1998 (18)	NR	Serology	Cases: higher seropositive rate; shared same antibody subtype [†]	B
Shirodaria et al, 1987 (21)	Yes	Serology	Multiple sclerosis and rheumatoid arthritis cases: higher titres [†]	B
Larsen et al, 1985 (17)	NR	Serology	All cases seropositive versus 84% of controls; cases: higher titres [†]	B
Sumaya et al, 1985 (24)	Yes	Serology, CSF	Cases: higher seropositive rate, titres [†]	B
Bray et al, 1983 (11)	Yes	Serology	Cases: higher seropositive rate, titres [†]	B
Sumaya et al, 1980 (23)	Yes	Serology	Cases: higher seropositive rate, titres [†]	B
Enbom et al, 1997 (15)	NR	Serology, CSF	No difference in seropositivity; cases: higher CSF antibody prevalence, titres	B
Bray et al, 1992 (12)	NR	CSF	Cases: higher antibody prevalence, titres in CSF [†]	C
Compston et al, 1986 (13)	No	Serology	No difference in seropositive rate	C
Nikoskelainen et al, 1972 (20)	NR	Serology	No difference in mean titres	C
Ellison et al, 1977 (14)	NR	Serology	DW2+ cases: higher mean titres	C
Kinnunen et al, 1990 (16)	NR	Serology	No difference in mean titres	C

*Coding of specimens; [†]Statistically significant. CSF Cerebral spinal fluid; NR Not reported

duce latent, recurrent infections (3). Epstein-Barr virus (EBV), in particular, is hypothesized to play an etiological role in this disorder. Acute infection leads to lifelong, latent infection of B lymphocytes (4). By adulthood, 90% of the population has been infected with EBV, as demonstrated by antibodies to this virus. Infection is often mild or not apparent in young children, while infection later in life may present as infectious mononucleosis (IM).

Acute neurological complications occur in 5.5% to 18% of those with IM, and include encephalitis, transverse myelitis and postinfectious encephalomyelitis (5,6). These may occur in the absence of typical manifestations of IM (7). This finding prompted unsuccessful efforts to identify EBV in the brains of MS patients (8). This lack of success may be due to insensitivity of detection methods, the fact that EBV does not have an etiological role, or that EBV merely initiates or perpetuates the disease process. In the present article, we evaluate the epidemiological evidence that EBV infection increases the subsequent risk of MS.

METHODS

Searches of the medical literature (1965 to 1999) were conducted with subject headings 'demyelination' or 'multiple sclerosis' AND 'Epstein-Barr virus' or 'infectious mononucleosis' or 'case-control' or 'cohort' or 'seroepidemiol*' or 'risk' using MEDLINE and the Cochrane Library to identify observational studies. Bibliographies of all articles retrieved were searched for additional articles not indexed in either database. Case reports, case series and reviews were excluded. Twenty-five observational studies evaluating EBV infection (or more specifically IM) and MS etiology were identified.

Studies were categorized by methodology into three groups – seroepidemiological, case-control and historical cohort studies. Ratings of methodological rigour were assigned using criteria derived from published guidelines for

the epidemiological study of MS (Appendix 1) (9,10). In each category, the criteria for a rating of 'A' were those considered to characterize an ideal study. The lowest rating assigned usually indicated a study lacking all of the ideal characteristics needed for an 'A' rating. Ratings between the highest and lowest ratings identified studies with some but not all of these ideal characteristics. Where necessary for complete evaluation of a study, prior publications referenced were retrieved.

RESULTS

Seroepidemiology

Fourteen seroepidemiological studies were identified (11-24). Two of three studies by Sumaya et al (22,24) included data from a single patient series; so, only the latter of the two studies was included in the review. There were no studies rated 'A', because the majority of investigators were not blind to subject status, and little information was provided concerning subject selection. Eight were rated 'B', and five were rated 'C' (Table 1).

Six of seven 'B' studies reported higher EBV seroprevalence among MS patients (Table 2) (11,17-19,21,23,24). All five 'B' studies measuring serum titres found them to be higher among MS cases (11,17,21,23,24). This included the results of Shirodaria et al (21), who measured only serum titres.

Compston et al (13) (a 'C' study) found no difference in seroprevalence. Of three 'C' studies measuring serum antibody titres, only Ellison et al (14) had positive findings (16,20). Titres were increased among human leukocyte antigen DW2+ MS patients (14).

The prevalence of antibodies in cerebral spinal fluid was measured by Bray et al (12) ('C' study) and Enbom et al (15) ('B' study). Enbom et al (15) found increased antibody prevalence and titres in cases. Another 'B' study by Sumaya et al (24) did not find increased titres among cases. The 'C'

TABLE 3
Case-control studies – Characteristics

Author (year)	Diagnostic criteria	Cases			Comparison group			
		Number	Exclusions	Source	Source	Number	Matching	Rating
Italian MS Study Group, 1989 (29)	McDonald and Halliday (26) – (revised) CDMS, CPMS	315 incident	Diagnosis >one year pre-admission	All newly diagnosed hospitalized MS patients	Neurological and non-neurological hospital patients without MS, patients with optic neuritis or autoimmune, psychiatric or ethanol-related disease; suffered from disease for longer than five years; similar age and sex as expected for cases	1975	Residence in same province for more than six months	A
Casetta et al, 1994 (27)	McAlpine (46) – CDMS	104 prevalent	Cognitive impairment	All cases in Ferrara, Italy	General population, non-MS hospital patients; excluded transitory CNS disorders not yet diagnosed, optic neuritis	150	Age ± three years, sex, residence	B
Gusev et al, 1996 (28)	McAlpine (46) – CDMS, CPMS	155 prevalent	Cognitive dysfunction	Consulting centre, hospital, surrounding area	Nonautoimmune neurological patients, ophthalmology patients, volunteers, medical students, non-blood relatives	169	Age ± five years, sex, residence	B
Marrie et al, 2000 (35)	Definite, probable	225	Prevalent demyelinating disease	General Practice Research Database (Secretary of State for Health, United Kingdom)	General Practice Research Database	900	Age ± two years, sex, physician practice	B
Poskanzer et al, 1980 (33)	Allison and Millar (47) – CPMS, CPOMS	81 prevalent	None	Orkney and Shetland Islands, Scotland	Random parish control; discontiguous control; spouses, first-degree relatives	NR	Age, sex ± parish where born and raised	B
Martyn et al, 1993 (31)	Acutely relapsing CDMS, acute optic neuritis, isolated demyelinating lesion	225 incident, prevalent	Age >50 years	Patients at National Hospitals for Nervous Disease, Queen Square or Maida Vale, Moorfields Eye Hospital (London, England)	Human leukocyte antigen DR2+ blood donors; subjects with other neurological or infectious/immunological processes from same hospitals	164	None	C
Souberbielle et al, 1990 (34)	Poser et al (36) – definite	153 incident (most)	None	Patients at Henri Mondor Hospital (Paris, France), 1974 to 1984	Random hospital patients without infectious, immunological and/or neurological disorders except intervertebral disc disease	153	Sex, year of birth	C
Operskalski et al, 1989 (32)	Definite, probable	145 prevalent	Dead	Some members of previously studied cohort	Friends without neurological disorders	145	Age ± five years, sex, race, birthplace, residence	D
Lenman and Peters, 1969 (30)	NR	50 prevalent	NR	Consecutive inpatients or outpatients	Non-neurological outpatients or inpatients from same ward	50	Age ± five years, sex, race	D

CDMS Clinically definite multiple sclerosis; CPMS Clinically probable multiple sclerosis; CPOMS Clinically possible multiple sclerosis; LSDMS Laboratory-supported definite multiple sclerosis; LSPMS Laboratory-supported probable multiple sclerosis; MS Multiple sclerosis; NR Not reported

study demonstrated higher cerebral spinal fluid antibody prevalence and titres among cases (12).

Most studies found increased EBV antibody seroprevalence and serum titres in MS patients (11,17-19,21,23,24).

Some studies included patients without MS as cases or failed to report diagnostic criteria. The studies frequently failed to blind laboratory procedures or describe their sources of cases and controls.

TABLE 4
Case-control studies – Methods and results

Author (reference)	Exposure ascertainment		Results	Rating
	Blinded*	Methods		
Italian Multiple Sclerosis Study Group, 1989 (29)	Yes	Trained interviewers	No difference in frequency of IM, odds ratio=0.62 (95% CI 0.22 to 1.80)	A
Casetta et al, 1994 (27)	No	Trained interviewers	No difference in frequency, age of onset of IM	B
Gusev et al, 1996 (28)	Yes	Trained interviewers	No difference in frequency	B
Marrie et al, 2000 (35)	Yes	Database review	Higher frequency of IM, odds ratio=5.5 (95% CI 1.5 to 19.7) [†]	B
Poskanzer et al, 1980 (33)	NR	Pretested questionnaire by trained interviewers	No difference in age at IM	B
Martyn et al, 1993 (31)	No	Interview by investigator involved with previous study; serology	In seropositive subjects with a history of IM, odds ratio=2.9 (95% CI 1.1 to 7.2); in subjects with IM before the age of 17 years, odds ratio=7.9 (95% CI 1.7 to 37.9) [†]	C
Souberbielle et al, 1990 (34)	No	Interview by investigator	No difference in frequency	C
Operskalski et al, 1989 (32)	NR	Self-administered questionnaire ± phone interview	Higher frequency of IM, odds ratio=17.0 (95% CI 2.0 to 81.8) [†]	D
Lenman and Peters, 1969 (30)	NR	Interview	No difference in frequency	D

*Blinded to subject status and/or study hypotheses; [†]Statistically significant results. IM Infectious mononucleosis; NR Not reported

TABLE 5
Historical cohort studies

Author (reference)	Source	Number	Diagnostic criteria	Relative risk	Rating
Haahr et al, 1995 (37)	Danish population registry: positive heterophil antibody tests 1968 to 1978, except 1975; and negative heterophil antibody tests 1968 to 1970, 1978	19,739	Allison and Millar (47) (modified) – definite, probable, latent probable	2.81	A
Lindberg et al, 1991 (38)	Hospital registry's heterophil antibody-positive infectious mononucleosis cases	494	Poser et al (36) – definite	3.7	A

Case-control studies

Nine case-control studies were identified (25-35). One 'A', four 'B', two 'C' and two 'D' ratings were assigned (Tables 3, 4). In all but one study, information was obtained through self-administered questionnaires or interviews, but only one assessed seropositivity. One study obtained information from a database with prospectively collected information (35).

The 'A' study by the Italian Multiple Sclerosis Study Group found no difference in the reported frequency of IM among cases and controls (29).

One of the 'B' studies found an increased risk of MS in subjects with a history of IM (35). The odds ratio (OR) was 5.5 (95% CI 1.5 to 19.7). The OR, when IM occurred after the age of 17 years, was 6.0 (95% CI 1.4 to 25.4). In this study, only 11 subjects of 225 cases and 900 controls had IM. While definitions of cases and controls were clearly described, the diagnostic criteria used were not previously established standard criteria such as those of Poser et al (36). The criteria used were created for the purposes of that study because of the limitations imposed by the information available in the database being used. None of the other 'B' studies detected a difference in the reported frequency of IM among cases and controls (27,28,33). Their sample sizes

ranged from 81 to 225. Neither of the other 'B' studies assessing age at occurrence of IM found a significant difference between groups (27,33).

Souberbielle et al (34) ('C' study) also found no difference in the reported frequency of IM. Martyn et al (31) ('C' study) enrolled cases with acutely relapsing MS, optic neuritis or other isolated demyelinating lesions. Past exposure was assessed using an interview and serology. In seropositive individuals reporting a history of IM, the OR was 2.9 (95% CI 1.1 to 7.2). The OR, when IM occurred before the age of 17 years, was 7.9 (95% CI 1.7 to 37.9).

There were two 'D' studies (30,32). Operskalski et al (32) found that cases were much more likely than controls to report a history of IM (OR 17.0, 95% CI 2.0 to 81.8). This study used prevalent cases without stating diagnostic criteria, and a study collaborator interviewed all subjects. Lenman and Peters (30) interviewed 50 consecutive inpatients and outpatients with MS, and 50 matched controls from the same ward. Diagnostic criteria were not described. There was no difference in the reported frequency of IM.

Statistically significant differences emerged only in case-control studies with weaker methodologies and in one 'B' study. The diagnostic criteria were adequate in most studies,

but prevalent cases were enrolled, raising the problem of selective survivor bias. Lack of blinding in some studies and the uncertain accuracy of recalled, self-reported diagnoses of IM are important sources of measurement bias.

Historical cohort studies

Two historical cohort studies were identified, both using adequate diagnostic criteria (37,38). Both were rated as 'A' (Table 5). Lindberg et al (38) assembled a cohort of 494 individuals with heterophil antibody (HA)-positive IM from a hospital registry, and linked it to subsequent hospital records and an MS register. On comparison of observed and expected MS rates, the estimated relative risk in IM patients was 3.7 (no CI given). Haahr et al (37) used a Danish registry comprising all patients with positive HA tests between 1968 and 1978 (except 1975), and all patients with negative HA tests in 1978 and 1968 to 1970. On comparison of observed and expected MS rates, the estimated relative risk among the HA-positive group was 2.8 (no CI given). The advantages of using registries include elimination of recall bias and reduction of selection bias.

DISCUSSION

There is geographic and temporal variation in MS incidence rates, and there is an apparent change in disease risk associated with migration between areas with differing risks of MS, depending on the age at migration (39). Migration studies have been interpreted to indicate the importance of exposure to an environmental factor in early life between the ages of 10 and 15 years (39). This has led to hypotheses that infections are etiological factors in MS and that the timing of these infections is important.

It has been argued that if MS is secondary to persistent viral infection or an immune reaction to a resident virus, it should be possible to identify the virus in the central nervous system at some phase of the disease, as has been the case in other chronic, progressive viral diseases (40). Others have tempered this argument, suggesting that in chronic infections, virus gene expression may be restricted, and traditional techniques may not be sensitive enough to detect viral material (41). Hilton et al (42) performed *in situ* hybridization with EBV-specific RNA probes on 21 plaques from 10 postmortem MS cases but were unable to detect a signal. Direct evidence of EBV infection of the CNS has not been demonstrated to date. However, in chronic infections, virus gene expression may be restricted, and traditional techniques may be insensitive for the detection of viral material (41).

Acute neurological complications are associated with IM (5,6). A single case series, consisting of five patients with primary EBV infection complicated by neurological involvement, described the development of classical MS in four patients and diffuse demyelinating disorder in the fifth (43). This argues for a role of EBV in the development of MS.

To date, evidence for an etiological role of EBV in MS exists largely in the epidemiological literature, which has

examined only the evidence of systemic, not CNS, EBV infection. The etiological role of EBV in MS was reviewed in the present paper using seroepidemiological case-control and cohort studies. The strongest epidemiological evidence was derived from a randomized controlled trial (experimental study). This sort of evidence is obviously not available or appropriate in the study of risk factors for MS. Observational studies are the only feasible way of studying most questions concerning risk. Cohort studies provide the strongest level of evidence available. These studies have the advantage of establishing exposure without the bias of already knowing the disease outcome, and can assess the relationship between exposure and many diseases. Unfortunately, these studies require large numbers of subjects and prolonged follow-up, and are expensive.

Case-control studies provide the next level of evidence. These studies are easier to conduct than cohort studies, and are particularly useful for the study of rare diseases but are susceptible to bias. These studies are strongest if incident cases are used, thus avoiding the risk of selective survivor biases.

Seroepidemiological studies document evidence of prior infection but cannot establish when an infection occurred, its severity or even whether it was clinically symptomatic or asymptomatic. These studies are more appropriate for generating, rather than testing, etiological hypotheses.

There was a large variation in the quality and type of evidence available, and this impeded the synthesis of the data. Differences in the reporting of data and the amount of raw data available in the studies precluded statistical combination of the data. Whether the occurrence of IM as a manifestation of EBV infection or the occurrence of any EBV infection has differential importance is unclear, and all studies did not address the same question in this respect.

While both well designed ('A') historical cohort studies and one case-control study ('B') found increased risks of MS in subjects with HA-positive IM, results from other studies were less convincing. In historical cohort studies, the subjects were identified only if they had a test confirming exposure status, while in case-control studies, they were identified by disease status. This may explain the differences in their results. Overall, the evidence is insufficient to accept or reject the hypothesis that EBV increases subsequent risk of MS. A recent review concluded that there was evidence to support a role of EBV in the etiology of MS. Ascherio and Munch (44) evaluated studies comparing EBV serology in MS patients with controls, but did not examine the characteristics of the studies in detail. They concluded that the summary OR of MS comparing EBV-seropositive individuals with EBV-seronegative individuals was 13.5 and that the strength of the association supported a role of EBV in MS. Several studies have demonstrated higher seroprevalence and higher antibody titres to other viruses, including measles, mumps and rubella (11,20,21). Elevated viral antibody titres have also been demonstrated in other disorders. One study, in particular, conducted by Shirodaria et al (21), demonstrated an increased rate of

seropositivity, as well as elevated titres in MS and rheumatoid arthritis patients, compared with controls. There were no differences between the MS and rheumatoid arthritis patients. Serological data alone would thus seem insufficient to support a role of EBV in MS.

Given the establishment of latent infections, the association of neurological complications with IM and the above results, a potential etiological role for this virus remains attractive, although difficult to study. EBV may be one of many factors capable of causing MS in a genetically susceptible individual and may be neither a necessary nor a sufficient cause (1,45). Exposure is likely to be highly prevalent

among subjects with and without MS, requiring large sample sizes to identify an effect. MS is relatively rare, probably with a long latent period between exposure and symptom onset, making it difficult to verify the temporal relationship between exposure and disease onset. It would be of interest to determine whether EBV infection alone or its clinical manifestation as IM is of more importance in MS risk. Does EBV interact with other exposures or genetic factors to cause MS? Further study, ideally using large samples of incident cases with blinded, trained interviewers using confirmatory sources for recalled data, is justified to explore these and other questions.

APPENDIX 1

Criteria for rating studies

Seroepidemiological studies

- A Clearly described case definition, including reference to established criteria, or detailed description of those used
Controls selected from an a priori defined study base
Laboratory investigators blinded to subject status
Statistical analysis specified and appropriate
- B Clear definitions of cases and controls, but not meeting criteria of A
Sources of cases and controls not reported
Blinding not reported or absent
Statistical analysis unspecified
- C Not meeting criteria of B

Case-control studies

- A Use of incident cases
Clearly described case definition
Controls selected from an a priori defined study base
Exposure ascertainment methods with interviewers blinded to subject status and/or study hypotheses
Exposure ascertainment described, and the same for cases and controls
Confirmatory source for recalled data
Inclusion of an etiologically relevant time period (stating the period of relevant exposure)
Statistical analysis specified and appropriate
- B Clear definitions of cases and controls, but using prevalent cases
Otherwise meeting criteria of A
- C Use of prevalent cases
Controls selected without regard to study base
Incomplete or unreported blinding of exposure ascertainment
Time period not etiologically relevant or poorly presented
No confirmatory source for recalled data
- D Not meeting any criteria of A, B or C

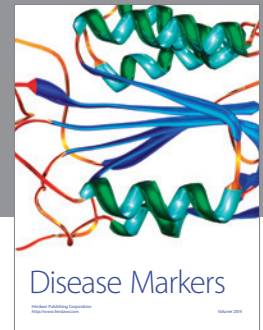
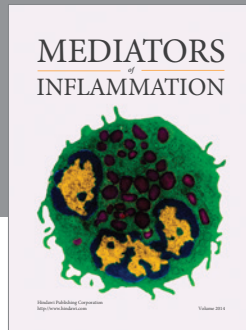
Historical cohort studies

- A Clearly defined cohort
Exposure of interest defined
Clearly described case definition
Reasonably complete case ascertainment (80%)
Statistical analysis estimating relative risk
- B Not meeting criteria of A

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