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Selective association of multiple sclerosis with infectious mononucleosis

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Previous studies have suggested an association between multiple sclerosis (MS) and infectious mononucleosis (IM) but data on the exact strength of this association or its selectivity have been conflicting. In this study we have evaluated the association between MS and a variety of common childhood infections and afflictions in a large population-based case-control study involving 2877 MS cases and 2673 controls in the Netherlands. We examined the frequency of different common infections and afflictions before the age of 25 and the age at which they occurred, using a self-administered questionnaire.

The Odds ratios (ORs) for the occurrence of a variety of clinically manifest common childhood infections including rubella, measles, chicken pox and mumps before the age of 25 for MS cases versus controls ranged between 1.14 and 1.42, values similar to those for irrelevant probe variables used to reveal recall bias. In contrast, the OR for clinically manifest IM in MS cases versus controls, corrected for demographic variables, was 2.22 (95% confidence interval 1.73 – 2.86; $P < 0.001$). The average age of onset of IM in the population of MS cases (16.5 years) did not differ from controls (16.8 years). Our data confirm previous much smaller studies to show that the risk for MS is significantly enhanced by prior IM, and extend those previous data by showing that this association is far stronger than with other common childhood infections or afflictions. *Multiple sclerosis* 2008; 14: 307–313. <http://msj.sagepub.com>

Key words: immunology; multiple sclerosis

Introduction

The most consistently reported association between MS and an infectious agent is that with Epstein–Barr virus (EBV). Already in the 1980s, it was noted that the worldwide distribution of multiple sclerosis (MS) coincides with areas in which delayed infection with common viruses such as EBV is prevalent [1]. Several studies have since examined the association of MS with infectious mononucleosis (IM). Infectious mononucleosis is the clinical manifestation of a pathologically strong immune response that tends to develop when primary infection with EBV is delayed until adolescence. Collectively, these studies based on cohorts of up to 300 MS cases have generated data that point to an association between MS and IM. As recently reviewed, however, the relative risk emerging from these studies ranged between 1 (no risk at all) up to 17 [2]. Also, it has

remained largely unclear to what extent an increased risk for MS after IM is specific for EBV, or also holds for delayed primary infection with other common viruses. Two recent studies on the association between MS and other common childhood infections for example, have produced conflicting results. Although a study by Bager *et al.* in Denmark involving 455 cases suggested no significant association between MS and prior measles, rubella, mumps, varicella, pertussis or scarlet fever [3], a case-control study involving 110 cases in Serbia suggested that several of these common infections were in fact also significantly more frequent in MS cases included in that study [4].

Several studies have suggested that the strength of an immune response against EBV is associated with the risk to develop MS. Direct examination of immune parameters have shown that levels of serum antibodies against EBV antigens are associated with

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MS [5], as is the strength of helper and cytotoxic T-cell responses to EBV antigens [6–8], and the more frequent presence of oligoclonal IgGs directed at EBV antigens in the cerebrospinal fluid [7]. Especially the immune response against the EBV-encoded nuclear antigen-1 has been found to highlight this association. That EBV infection has a particular relevance to MS has been further supported by the observations that essentially all MS patients are seropositive for EBV, whereas in the general adult population, 5–15% seronegativity is generally found [1] and in pediatric MS such a consistent link with EBV has also been reported [9].

In the present study, we conducted a population-based case-control study to assess the relative risk for MS after prior IM and to examine whether such an association is selective for delayed EBV infection or perhaps, also applies to other common childhood infections.

Materials and methods

To perform the population-based case-control study, we approached 4000 individuals with MS and 6000 control individuals. Multiple sclerosis cases were derived from a random selection of MS patients who held a membership of the Dutch MS Association (MSVN). Population controls were randomly selected from the household telephone subscription registry of a national Dutch telephone company (KPN). Considering the generally two-fold over-representation of women over men in the MS population, we randomly invited 2000 men and 4000 women from the 6000 control households to participate. In case an invitation was sent to the woman in the household that consisted of only of an adult man, the man was invited to participate, and *vice versa*. To avoid generating any specific attention to either MS or any particular childhood disease, the study presented to controls was referred to as 'Childhood diseases and present health status'. The study was presented to MS cases as 'Childhood diseases and MS'.

All MS cases were invited by the MSVN to participate in the study by completing a self-administered questionnaire. Controls were invited directly by the authors to participate in the study. All participants were asked to send the completed questionnaire anonymously to our institute. In case an individual declined the invitation, they were asked to send the blank questionnaire back and to give a reason for declining. The self-administered questionnaire covered sociodemographics (age, sex, educational level, area of residence), childhood diseases, factors associated with viral exposures, hormonal influences in women, disease characteristics, comorbidity and general health.

Questions on childhood diseases included some randomly chosen afflictions and several common viral infections including chicken pox, measles, mumps, rubella and IM (in Dutch referred to as 'ziekte van Pfeiffer'). Afflictions with no known or suggested relationship to MS were included as so-called probe variables, relevant to establish the level of recall bias among cases as compared with controls. These probe variables included the occurrence of a broken arm, concussion and tonsillectomy. For each disorder, individuals were asked to report to the best of their knowledge whether or not they had experienced the affliction or infection before the age of 25, choosing between 'yes', 'no' and 'not sure'. In case of doubt, individuals were encouraged to consult family members for additional information. In case of a positive answer, individuals were asked to indicate at what age the disorder was noticed for the first time. Information on comorbidity was assessed by a Dutch validated standardized instrument on chronic diseases used by the Central Bureau of Statistics in the Netherlands for monitoring chronic diseases in the general population.

The extent to which the control population was representative for the general population was studied by comparing sex-specific comorbidity data of our control group standardized for the age-distribution of the general population to published reference data for the general population. By calculating 95% confidence intervals (CIs) for our control population, we examined the occurrence of statistically significant differences in morbidity between our study population and the general population.

Since we aimed to limit the analysis to childhood and adolescent disorders occurring before the age of 25, all subjects under the age of 25 were excluded from the analysis. To evaluate recall bias and the association between MS and before the age of 25, we performed multivariate unconditional logistic regression methods to calculate odds ratios (ORs) and their 95% CIs, controlled for possible confounding factors including age, sex, educational level and place of residence.

Results

Response

As summarized in Table 1, of the population of invited individuals, 2877 MS cases (71.9%) and 2673 controls (44.5%) participated by returning a completed questionnaire. Higher response rates for individuals with MS most likely reflect the fact that they had been invited through the patient society of which they were all members. The overall response rate of all 10 000 invitees was 55.5%.

Table 1 Response rates for MS cases and population controls

	MS cases	Population controls
Invited to participate	4000	6000
Completed a questionnaire	2877 (71.9%)	2673 (44.5%)
Declined to participate	10 (0.3%)	64 (1.1%)
Died	13 (0.3%)	29 (0.5%)
Mail undeliverable	9 (0.3%)	41 (0.7%)
Declined because of illness	3 (0.1%)	4 (0.1%)
Nonresponders	1088 (27.2%)	3189 (53.2%)

The small number of invitees among cases and controls who explicitly declined participation and sent back their questionnaire (0.3 and 1.1%, respectively) in most cases did not explain their objection.

Control population

For a large case-control study such as the present one, it is of importance to verify that the control population is in fact representative for the general population with regard to their health status. To validate this, self-reported chronic conditions within the control population were standardized for age distribution and compared to reference data for the general population in the Netherlands, separately evaluated for both sexes. The control population reported slightly more chronic conditions as compared with the available reference data for the general population. However, the greatest difference

between the lower bound of the 95% CI and the point prevalence of the general population was only 4.3% for perinasal, frontal or maxillary sinusitis for women. On the basis of the extensive data set obtained (not shown), we conclude that the general health of the control group in this study was adequately representative for the general population to serve as a reference control.

General characteristics of MS cases and controls

Forty percent of the MS cases reported to have chronic-progressive MS, while 33% reported to have relapsing-remitting MS. Of the remaining cases, the clinical subtype of MS was unknown. Mean age at the final clinically definite diagnosis of MS reported by cases was 37.4 years. General characteristics of MS cases and controls are summarized in Table 2. Overall, the population of 2877 people with MS included slightly more people under the age of 65, more women and somewhat more people from the northern parts of the Netherlands. The limited variations in these demographic factors were considered as potential confounding factors in the multivariate analysis.

Recall bias

An obvious problem in evaluating self-reported health parameters is the issue of recall bias. Being confronted with their serious disease, people with

Table 2 General characteristics of MS cases and population controls

Variable	Categories	MS cases (n = 2877)%	Population controls (n = 2673)%	P-value ^a
Age	15–24	1.2	4.2	<0.001
	25–34	13.0	21.5	
	35–44	28.5	21.2	
	45–54	29.6	20.4	
	55–64	16.6	14.8	
	65 and older	10.3	17.5	
Sex	Missing	0.8	0.4	<0.001
	Men	29.6	38.0	
	Women	69.5	61.4	
Area of residence	Missing	0.9	0.6	0.003
	West	38.4	36.8	
	North	12.5	10.1	
	East	16.7	18.5	
	Middle	9.2	9.1	
	South	22.7	24.5	
Education level	Missing	0.5	1.0	<0.001
	Primary	30.7	29.7	
	Secondary	41.7	40.7	
	Vocational	18.5	24.5	
	College/University	7.8	4.3	
	Missing	1.3	0.8	

^a χ^2 test.

MS are more likely than controls to reflect on and recall past events that could help explain the development of MS, including certain childhood afflictions. At the time this study was performed, no significant public awareness existed with regard to the association of EBV infections with MS any more than with regard to other infectious agents or environmental factors. To examine the extent of recall bias in our study, we evaluated the association between MS and the different probe variables that had intentionally been introduced for this purpose. Despite the fact that the pathogenic process of MS has not yet been fully clarified, several factors can be safely assumed to be unrelated to the disease, also supported by previous epidemiological studies. To the best of our knowledge, no association has been reported between MS and incidences of broken limbs, tonsillectomy or concussion. The latter two events have been subject of studies to show that there are no detectable associations [10,11]. On the basis of these probe variables, the extent of recall bias in our study can be estimated.

We found that with regard to the probe variables, cases more often than controls reported a 'not sure' answer. Several different explanations can be given for this phenomenon. Multiple sclerosis cases might be more inclined than controls to think that they might have had a specific affliction without an immediate recollection of the occurrence. Alternatively, MS cases might be inclined to more carefully consider their answer and to opt for a 'not sure' option, in case of even the slightest doubt. In line with this, slightly positive associations between MS and the probe variables were almost consistently found. After accounting for differences in age, sex, educational level and area of residence by logistic regression, probe variables yielded ORs between 1.10 and 1.25, as shown in

Table 3, clearly illustrating some level of recall bias in the data set.

To control for differential recall bias between cases and controls in the specific association of MS with IM as well as with the other childhood infections, we performed regression analyses based on four different models. In the first model univariate regression analysis was performed on the association between MS and childhood infections using the three different categories of answers ('yes', 'no' and 'not sure'). In the second model the same data set was analysed by multivariate regression analysis. In the third model, multivariate regression analysis was again used but now with the answers divided into two categories: 'yes' on one hand and 'no' or 'not sure' on the other. In the final model a multivariate analysis of the association with IM was performed using the three answer categories 'yes', 'no' and 'not sure' separately, together with all probe variables for which the answers had been categorized into the emphatic answers ('yes' or 'no') on one hand and the uncertain answers ('not sure') on the other. In this final model, the effect of the tendency to provide uncertain answers was incorporated as a possible confounding personality trait.

Viral childhood diseases and MS

Using the above models and considerations, the association between MS and the different afflictions was calculated, as summarized in Table 3. In this table, the ORs and their 95% CIs (95% CI) were corrected for age, sex, region of residence and level of education. The analyses indicate that the association between MS and IM is statistically significant ($P < 0.001$), irrespective of the model used to perform regression analysis. The risk for MS is

Table 3 ORs and 95% CI of various childhood diseases, controlled for age, sex, educational level and area of residence

Childhood disease	MS cases (<i>n</i> = 2821)%	Population controls (<i>n</i> = 2550)%	OR (95% CI)	<i>P</i> -value
A broken arm	14.5	13.5	1.10 (0.93–1.30)	0.271
Mumps	51.3	49.0	1.14 (1.01–1.30)	0.023
Bronchitis	16.3	14.4	1.17 (1.00–1.37)	0.051
IM	8.0	4.1	2.22 (1.73–2.86)	<0.001
Sibling with IM	8.4	6.3	1.41 (1.13–1.75)	0.002
Concussion	23.7	20.0	1.23 (1.07–1.41)	<0.001
Rubella	32.6	26.1	1.31 (1.14–1.49)	<0.001
Hay fever/pollinosis	8.1	7.1	1.28 (1.03–1.58)	0.014
Tonsillectomy	49.7	42.2	1.25 (1.11–1.40)	<0.001
Whooping cough/pertussis	12.8	10.4	1.22 (1.02–1.47)	0.016
Measles	77.2	73.8	1.42 (1.17–1.72)	<0.001
Cold sore	28.4	27.1	1.09 (0.95–1.26)	0.214
Pneumonia	8.9	9.6	0.92 (0.76–1.11)	0.491
Chicken pox/varicella	66.3	62.3	1.22 (1.04–1.43)	0.033

increased 2.2-fold by a previous episode of IM (OR = 2.22; 95% CI 1.73–2.86). No data were available on the severity of IM in either cases or controls, precluding an analysis of the possible relationship between this severity and MS. In line with the notion that delayed EBV infection is at least in part determined by factors of general hygiene in the immediate environment during childhood, also the frequency at which MS cases reported to have had a sibling with IM was somewhat higher (OR = 1.41; 95% CI 1.13–1.75).

The association of MS with other common childhood infections or afflictions was consistently much lower than for IM. This is illustrated in the data set given in Table 3 for common viral infections during childhood including mumps (OR = 1.14; 95% CI 1.01–1.30), chicken pox (OR = 1.22; 95% CI 1.04–1.43), rubella (OR = 1.31; 95% CI 1.14–1.49) and measles (OR = 1.42; 95% CI 1.17–1.72). Similar ORs were found for common bacterial infections.

Since the occurrence of IM is age linked, we also compared the age reported by either MS cases or controls for the occurrence of IM, as well as for the other infections and afflictions. As summarized in Table 4 no significant difference was found for the age at which IM manifested itself in MS cases (16.5 ± 4.5 years) as compared to controls (16.8 ± 3.8 years). Such differences were also not recorded for any of the other variables analysed.

Discussion

The present study confirms that IM significantly increases the risk for MS, and extends previous data on this subject by providing evidence that this association is much stronger than with any other common childhood infection. The strength of our study not only relies on numbers (2821 MS cases

and 2550 controls over the age of 25) but also on the direct comparison of data on IM with those on other childhood infections, as well as on events that can be considered irrelevant to MS. Such irrelevant events such as concussion, tonsillectomy and a broken arm can be used as probe variables in the analysis to assess recall bias, which addresses the most obvious drawback of the methodology we applied. The first two conditions yielded statistically significant ORs of 1.23 and 1.25, respectively, while the OR for a broken arm was 1.10. These numbers thus provide an estimate of the level of recall bias using the present methodology. We chose not to use these numbers to mathematically adjust the rest of the data, to allow for a direct comparison of the current data set with previously reported ORs, which were similarly not adjusted for recall bias [2]. Yet, it should therefore be kept in mind that even statistically significant OR's of up to around 1.25 probably have little biological relevance to the disease process.

Previous studies on much smaller groups of patients have already suggested the presently confirmed association between IM and MS. A recent meta-analysis by Ascherio *et al.* of collective data reported up to 2006 [2] generated a relative risk for MS after IM of 2.3 with a 95% CI of 1.7–3.0. Our data indicate a relative risk of 2.22 with a 95% CI of 1.73–2.86. The striking similarity between these numbers appears to lend further credence to their validity. The relative risk for MS in association with any other childhood infection, including both viral and bacterial infections, ranged between 1.1 and 1.4. These values are similar to those of the irrelevant probe variables used to reveal recall bias. While some of those associations were in fact statistically significant, they are markedly weaker than the association with IM. We therefore conclude that the association between MS and IM is selective.

Table 4 Mean age at occurrences of childhood diseases for MS cases and controls

Childhood disease	MS cases mean age \pm SD	Population controls mean age \pm SD	P-value ^a
A broken arm	11.4 \pm 5.4	11.3 \pm 5.4	0.692
Mumps	6.9 \pm 3.4	7.3 \pm 3.5	0.122
Bronchitis	9.1 \pm 6.6	9.4 \pm 6.5	0.584
IM	16.5 \pm 4.5	16.8 \pm 3.8	0.816
Sibling with IM	19.0 \pm 7.9	18.7 \pm 8.3	0.476
Concussion	11.8 \pm 5.7	12.0 \pm 5.3	0.559
Rubella	6.9 \pm 4.1	6.8 \pm 3.8	0.411
Hay fever/pollinosis	14.6 \pm 5.3	15.2 \pm 7.1	0.283
Tonsils removed	9.3 \pm 7.5	9.4 \pm 8.1	0.791
Whooping cough/pertussis	5.4 \pm 3.4	5.5 \pm 2.9	0.718
Measles	5.2 \pm 2.4	5.3 \pm 2.5	0.847
Cold sore	13.0 \pm 5.0	13.2 \pm 5.0	0.454
Pneumonia	9.6 \pm 7.0	10.5 \pm 7.2	0.144
Chicken pox/varicella	5.7 \pm 3.2	5.6 \pm 3.2	0.791

^aAnalysis of variance.

While delayed primary EBV infection has been implicated as a risk factor in the development also of other immune-mediated disorders, its relationship to MS remains striking. This is particularly evident from the fact that essentially without exception, all MS patients are seropositive for EBV pointing to the virus as a necessary cofactor in disease [1]. Clearly, EBV infection does not cause MS. In our current study population, IM manifested itself at an average age between 16 and 17, which is markedly different from the average age of 30 at which clinical signs of MS tend to manifest themselves. Other studies have clarified that no EBV can be detected in MS lesions [12], and any temporal relationship between active EBV infection or reactivation and clinical features of MS is weak at best [13]. Thus, not the virus itself but rather the immune responses that develop upon infection are likely to be crucial factors to explain the association.

The issue raised by these findings is therefore how an EBV-activated immune response can be linked to an inflammatory process that appears to be limited to the central nervous system (CNS), and in particular to CNS myelin, in the apparent absence of viral antigen. Some studies have shown that sufficient structural similarity exists between certain EBV protein sequences and myelin peptides to allow distinct helper T-cell clones or antibodies to cross-react between the two [14,15]. Yet, evidence is lacking that such cross-reactivity would be functional at the level of intact protein antigens, rather than the synthetic peptides used in the experimental systems. Also, no evidence exists to demonstrate that cross-reactivity of individual T-cell receptor clonotypes between EBV peptides and myelin peptides reaches any biologically significant level in the context of the polyclonal T- and B-cell responses that develop upon infection *in vivo*.

We currently favor an explanation based on previous data that EBV-infected B-cells protect themselves from virus-induced apoptosis by *de novo* expression of the stress protein alpha B-crystallin [16]. As a consequence, this protein becomes presented via MHC class II molecules of infected B cells. In the absence of any natural mechanism to secure immunological tolerance for alpha B-crystallin in humans, the antigen can thus activate polyclonal helper T cell and antibody responses which are indeed readily detectable in adult humans including MS patients [17,18]. It is well conceivable that later on in life, this response becomes instrumental in the inflammatory reaction in the CNS that causes MS, since myelin-derived alpha B-crystallin becomes locally presented again at the earliest stages of lesion formation in MS [19]. As a result of the pre-existing EBV-linked memory response against this antigen, it could thus become a major target of the local recall response triggered by CNS myelin [17]. In this

view, EBV infection could well be associated with MS as a necessary cofactor since it induces life-long immunity against an antigen that is shared between EBV-infected B-cells and stressed CNS oligodendrocytes.

It remains to be conclusively established which mechanism explains why EBV infection appears to represent a necessary step in the ultimate development of MS. The present data support the notion that the strength of an immune response triggered by EBV contributes to MS. Importantly, they also indicate that this association is selective and does not apply to other common viral or bacterial infections during childhood. The accumulated data therefore, prompt further studies to clarify which molecular mechanisms link the immune response to a natural infection of humans with EBV to the subsequent development of chronic inflammatory damage to the CNS.

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