Multiple Sclerosis After Infectious Mononucleosis

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Background: Infectious mononucleosis caused by the Epstein-Barr virus has been associated with increased risk of multiple sclerosis. However, little is known about the characteristics of this association.

Objective: To assess the significance of sex, age at and time since infectious mononucleosis, and attained age to the risk of developing multiple sclerosis after infectious mononucleosis.

Design: Cohort study using persons tested serologically for infectious mononucleosis at Statens Serum Institut, the Danish Civil Registration System, the Danish National Hospital Discharge Register, and the Danish Multiple Sclerosis Registry.

Setting: Statens Serum Institut.

Patients: A cohort of 25 234 Danish patients with mononucleosis was followed up for the occurrence of multiple sclerosis beginning on April 1, 1968, or January 1 of the year after the diagnosis of mononucleosis or after a negative Paul-Bunnell test result, respectively, whichever came later and ending on the date of multiple scle-

rosis diagnosis, death, emigration, or December 31, 1996, whichever came first.

Main Outcome Measure: The ratio of observed to expected multiple sclerosis cases in the cohort (standardized incidence ratio).

Results: A total of 104 cases of multiple sclerosis were observed during 556 703 person-years of follow-up, corresponding to a standardized incidence ratio of 2.27 (95% confidence interval, 1.87-2.75). The risk of multiple sclerosis was persistently increased for more than 30 years after infectious mononucleosis and uniformly distributed across all investigated strata of sex and age. The relative risk of multiple sclerosis did not vary by presumed severity of infectious mononucleosis.

Conclusions: The risk of multiple sclerosis is increased in persons with prior infectious mononucleosis, regardless of sex, age, and time since infectious mononucleosis or severity of infection. The risk of multiple sclerosis may be increased soon after infectious mononucleosis and persists for at least 30 years after the infection.

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D ESPITE MANY YEARS OF IN-
tensive research, the causes of multiple sclerosis (MS) remain unknown. The prevailing view is that MS is an autoimmune disorder that results from various environmental factors acting in a genetically susceptible host.[1] Different lines of evidence have implicated infection with the Epstein-Barr virus (EBV) in the development of MS. Investigations have shown that levels of certain EBV antibodies correlate with MS disease activity[2,3] and are predictive of MS risk in apparently healthy individuals.[4-6] Because more than 90% of healthy adults are infected with EBV, infection alone is clearly insufficient to cause MS. Age at primary infection and the host's immunological response may be modifiers of the EBV-associated risk of MS. Accordingly,

infectious mononucleosis, usually caused by EBV infection in adolescence, has been associated with an increased risk of MS in small cohort studies and, less compellingly, in case-control studies.[7] However, the association between mononucleosis and the risk of MS remains poorly characterized with respect to sex and age at and time since mononucleosis. Because such information is pertinent to understanding the association, we assessed the risk of MS in a cohort study of patients diagnosed as having mononucleosis.

METHODS

PATIENTS AND REGISTERS

We established a cohort of persons tested serologically during the period 1940 to 1988 for EBV-related mononucleosis by the modified Paul-Bunnell reaction for heterophile antibodies at

Statens Serum Institut in Denmark.[8,9] Specifically, all individuals with positive test results (ie, Paul-Bunnell reaction positive at titers of 1/32) were considered to have EBV-related mononucleosis. We also enrolled a subset of those with negative test results (ie, Paul-Bunnell reaction negative at titers of 1/32) in the same period as a comparison group to assess access to testing.[9] Using information on name, sex, and date of birth recorded for each individual, we linked the cohort with the Danish Civil Registration System to obtain the personal identification number, unique to all Danish citizens alive on April 1, 1968, and later, as well as the vital status for these individuals.[10] Personal identification numbers were found for 81% of the records sought. Using the personal identification number as key, we linked the cohort to the Danish Multiple Sclerosis Registry[11] to obtain information on MS occurring in the cohort.

To evaluate the impact of mononucleosis severity, we reasoned that hospitalized patients would represent the most ill subset of mononucleosis cases. We therefore constructed another mononucleosis cohort based on the Danish National Hospital Discharge Register, which contains information on all discharge diagnoses of patients admitted to Danish hospitals since 1977.[12] The cohort comprised all individuals registered with a diagnosis of mononucleosis (*International Classification of Diseases, Injuries and Causes of Death, Eighth Revision* [1977-1993] code 075 and *10th Revision* [1994-1996] code DB27) during the period 1977 to 1996. Using the personal identification number as key, we obtained information on vital status and MS diagnosis from the Danish Civil Registration System and the Danish Multiple Sclerosis Registry, respectively.

DATA ANALYSIS

Follow-up of the patients with mononucleosis began on April 1, 1968, or January 1 of the year after the diagnosis of mononucleosis or after a negative Paul-Bunnell test result, respectively, whichever came later and ended on the date of MS diagnosis, death, emigration, or the date December 31, 1996, whichever came first. For persons with more than 1 test result recorded, follow-up started January 1 of the year after the positive test result or the most recent negative test result. The standardized incidence ratio (SIR) of MS in the cohort was expressed as the ratio of observed to expected cases based on disease patterns in the whole population. The expected number of MS cases was estimated as the sum of age-, sex-, and period-specific population-based incidence rates of MS in Denmark available from the Danish Multiple Sclerosis Registry, multiplied by correspondingly stratified person-years at risk. We estimated SIRs for MS stratified by sex, age at and time since the Paul-Bunnell test, and attained age, calculating 95% confidence intervals (CIs) for the SIRs, assuming a Poisson distribution of the observed cases. The MS risks were also compared between the cohorts with positive and negative Paul-Bunnell test results by Poisson regression analyses.[13]

Diagnosis of MS was used as the end point. However, because of the possible passage of several years between onset and diagnosis of MS, we conducted a parallel series of analyses using debut of MS symptoms as the end point. In modeling the incidence rate of debut symptoms of MS, we corrected for delayed reporting of MS symptoms adapting the method described by Esbjerg et al.[14] (Details on the calculation of variance and the CIs are available from us.) In all analyses, $P<.05$ was considered statistically significant.

RESULTS

A total of 104 cases of MS observed vs 45.91 expected in the cohort of individuals who had positive Paul-Bunnell test results corresponded to an SIR of 2.27 (95% CI, 1.87-2.75) (**Table**). The risk of MS among hospitalized patients with mononucleosis was also increased (SIR, 2.50; 95% CI, 1.65-3.80) based on 22 observed cases of MS. With 68 observed cases, the MS risk in the cohort of persons who had negative Paul-Bunnell test results was not statistically significantly different from the risk prevailing in the general population (SIR, 1.23; 95% CI, 0.97-1.56). Compared with the cohort with negative Paul-Bunnell test results, the risk of MS was increased in the cohort with positive Paul-Bunnell test results (relative risk [RR], 1.71; 95% CI, 1.23-2.36), adjusted for calendar period, sex, attained age, and age at Paul-Bunnell testing.

The MS risk was increased in both men and women (Table). In the positive vs negative Paul-Bunnell test result cohort comparison, the RR of MS appeared somewhat higher for men (RR, 2.33; 95% CI, 1.42-3.81) than for women (RR, 1.41; 95% CI, 0.94-2.10), although this difference was not statistically significant (*P* for homogeneity=.12), adjusting for calendar period, sex, attained age, and time since the Paul-Bunnell test.

Elevated SIRs for MS were observed for all ages at mononucleosis, except those 30 years or older, whether MS diagnosis (Table) or MS debut was used as the end point (data not shown). The SIR for MS diagnosis appeared to be inversely correlated with age at mononucleosis (*P* for trend=.03) (Table), but this trend was not seen in analyses of MS debut symptoms (*P* for trend=.18). No evidence was found that prior mononucleosis was associated with the risk of MS with onset in any particular age interval whether MS diagnosis (Table) or MS debut was the end point (data not shown).

When MS diagnosis was used as the end point, increased risk appeared 10 years or more after mononucleosis occurred and remained increased for more than 3 decades (Table). When MS debut was used as the end point, risk was increased in every 5-year period from the mononucleosis diagnosis (data not shown).

COMMENT

We followed up more than 25 000 persons with mononucleosis for more than 550 000 person-years and observed a more than 2-fold increased risk of MS. Whereas the observed increased MS risk as such is consistent with previous studies,[7] the magnitude of our study allowed us to characterize the association in detail. With the exception of 1 case-control study in women,[15] such information has not been reported in previous studies or the studies were too small for stratified analyses to be statistically meaningful.

In the present study, mononucleosis was associated with an increased RR of MS in both men and women. This observation is consistent with findings previously presented in a small subset of the present cohort (16 observed MS cases).[16] Thus, the mechanisms that govern the mononucleosis-MS association seem to act in both sexes.

A susceptibility window for exposure to an MS agent has been proposed.[17] Primary EBV infection in childhood typically entails no or mild symptoms but in ado-

Table. SIRs (With 95% CIs) for Multiple Sclerosis in Persons With Positive and Negative Reactions on the Paul-Bunnell Test

	Positive Reaction			Negative Reaction		
	Person-Years of Follow-up	Observed No. of Cases	SIR (95% CI)	Person-Years of Follow-up	Observed No. of Cases	SIR (95% CI)
Overall	556 703	104	2.27 (1.87-2.75)	711 225	68	1.23 (0.97-1.56)
Sex						
Male	300 724	51	2.64 (2.01-3.47)	357 984	23	1.11 (0.74-1.67)
Female	255 979	53	1.99 (1.52-2.61)	353 241	45	1.30 (0.97-1.75)
P for homogeneity			.15			.52
Age when Paul-Bunnell test was performed, y						
<10	30 179	6	3.08 (1.38-6.85)	119 238	8	1.23 (0.62-2.47)
10-14	75 019	18	2.99 (1.88-4.74)	76 277	10	1.72 (0.93-3.20)
15-19	252 364	54	2.48 (1.90-3.23)	152 324	15	1.14 (0.68-1.88)
20-29	170 090	24	1.72 (1.15-2.57)	185 423	23	1.28 (0.85-1.93)
≥30	29 052	2	0.91 (0.23-3.65)	177 963	12	1.02 (0.58-1.79)
P for trend			.03			.51
Time since Paul-Bunnell test, y						
<5	75 694	3	1.15 (0.37-3.58)	114 098	5	0.89 (0.37-2.13)
5-9	95 826	6	1.02 (0.46-2.28)	133 999	9	1.03 (0.54-1.98)
10-14	93 076	25	3.03 (2.04-4.48)	130 114	13	1.20 (0.69-2.06)
15-19	79 562	24	2.89 (1.94-4.31)	111 038	14	1.40 (0.83-2.37)
20-24	66 188	18	2.39 (1.50-3.79)	80 659	13	1.72 (1.00-2.96)
25-29	55 722	11	1.71 (0.95-3.09)	67 039	6	0.91 (0.41-2.04)
≥30	90 634	17	2.46 (1.53-3.96)	74 278	8	1.35 (0.67-2.70)
P for homogeneity			.09			.80
Attained age, y						
<20	43 700	2	4.65 (1.16-18.52)	74 481	1	2.00 (0.28-14.29)
20-24	69 968	8	2.48 (1.24-4.97)	71 050	2	0.64 (0.16-2.57)
25-29	82 985	11	1.76 (0.98-3.18)	87 821	11	1.65 (0.91-2.98)
30-34	79 907	20	2.49 (1.61-3.86)	90 992	8	0.85 (0.43-1.70)
35-39	70 953	24	3.03 (2.03-4.51)	83 768	12	1.23 (0.70-2.16)
40-44	62 361	16	2.04 (1.25-3.32)	75 304	14	1.41 (0.84-2.39)
45-49	51 776	10	1.64 (0.88-3.04)	62 224	9	1.19 (0.62-2.29)
≥50	95 053	13	2.13 (1.24-3.68)	165 585	11	1.31 (0.73-2.37)
P for homogeneity			.82			.65

Abbreviations: CI, confidence interval; SIR, standardized incidence ratio.

lescence often manifests as infectious mononucleosis.[18] Ambiguous results were obtained in analyses of MS risk by age at mononucleosis diagnosis. Using MS diagnosis as the end point, MS risk was inversely correlated with age at mononucleosis diagnosis, but when using MS debut as the end point, this was not observed. In contrast, an increasing risk with increasing age at mononucleosis diagnosis was observed in a small US-based comparison.[15] Although alternative interpretations are possible, our data provided little support for a particular MS susceptibility age window for mononucleosis.

We also assessed MS risk by time since having had mononucleosis. When MS diagnosis was considered, the elevated MS risk became statistically significant only 10 years or more after a mononucleosis diagnosis. Using MS debut as the outcome, however, RR was homogeneous from the period less than 5 years after a mononucleosis diagnosis and onward. The interpretation of these findings is challenging because of the natural history of MS. Assuming that environmental factors are crucial to its development, the period from exposure to MS diagnosis would be expected to correspond to an incubation period. This could not be established on a relative scale in the present analyses but would be apparent in an abso-

lute risk analytical approach. Notably, the elevated MS risk was observed continuously for more than 3 decades after the diagnosis of mononucleosis, which may indicate that mononucleosis changes the immunological status and confers a permanent excess in MS risk. In this regard, Sauce et al[19] have recently shown that mononucleosis confers an apparently permanent immunological alteration in the host, the consequences of which remain to be established.

Other possible explanations for the uniform MS risk pattern across age, sex, and time since diagnosis of mononucleosis include the possibility that, rather than or in addition to the EBV infection itself, the increased MS risk after a mononucleosis diagnosis reflects shared risk factors related either to late timing of the primary EBV infection or to a possibly genetically determined immunological propensity to react with infectious mononucleosis on primary EBV infection. High social class and correlates hereof have previously been considered risk factors for both mononucleosis[18] and MS.[20] However, no association between social class and MS in Denmark was observed by Koch-Henriksen[21] or Bager et al,[22] the latter of whom found no evidence that MS risk varied by family structure in a cohort study of 1.9 million Danes. Analo-

gously, one could postulate a common genetic predisposition to both mononucleosis and MS. Although an association between MS and certain HLA types (especially HLA-DR2) have been established,[23] no such relationship has yet been observed for infectious mononucleosis.[24]

The population-based setting in addition to the size of the cohort, the long follow-up time, and the unbiased ascertainment of both exposure and outcome by means of high-quality registries are among the strengths of our study. Furthermore, all MS cases were ascertained in the Danish Multiple Sclerosis Registry. The registry was established in 1956 and has since collected information on Danish MS patients; it has an estimated completeness exceeding 90%.[11] We also validated our findings for the cohort with positive Paul-Bunnell test results in a hospitalized mononucleosis cohort, a group likely to have severe mononucleosis. The MS risk estimates were similar between the cohorts, indicating that severity of symptoms did not affect risk much.

Evaluations of the sensitivity and specificity of the Paul-Bunnell test for heterophile antibodies have demonstrated relatively low sensitivity and high specificity.[25,26] Importantly, however, the low sensitivity of the Paul-Bunnell test presumably renders our results for the cohort with positive Paul-Bunnell test results conservative when compared with a possibly overestimated risk for the cohort with negative Paul-Bunnell test results, and thus hardly can explain the observed association.

To summarize, we observed a more than 2-fold increased risk of MS after mononucleosis apparent for up to 30 years of observation and uniformly distributed across strata of age and sex. This absence of variation in MS risk may reflect a permanent change in immunological status, which confers an excess in MS risk, a hypothesis that needs to be explored further.

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