

## ORIGINAL ARTICLE

# Characteristics of Hodgkin's Lymphoma after Infectious Mononucleosis

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## ABSTRACT

**BACKGROUND**

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Infectious mononucleosis–related Epstein–Barr virus (EBV) infection has been associated with an increased risk of Hodgkin's lymphoma in young adults. Whether the association is causal remains unclear.

**METHODS**

We compared the incidence rates of Hodgkin's lymphoma in two population-based Danish cohorts of patients who were tested for infectious mononucleosis: 17,045 with serologic evidence of having had acute EBV infection, and 24,614 with no such evidence. We combined the cohort of patients who had serologically verified infectious mononucleosis with a cohort of 21,510 Swedish patients with infectious mononucleosis (combined total, 38,555). Biopsy specimens of Hodgkin's lymphomas occurring during follow-up in this combined cohort were tested serologically for the presence of EBV. Using this information, we modeled the relative risk of EBV-negative and EBV-positive Hodgkin's lymphoma in different periods after the diagnosis of infectious mononucleosis and estimated the median incubation time for mononucleosis-related EBV-positive Hodgkin's lymphoma.

**RESULTS**

Only serologically confirmed infectious mononucleosis was associated with a persistently increased risk of Hodgkin's lymphoma. Sixteen of 29 tumors (55 percent), obtained from patients with infectious mononucleosis, had evidence of EBV. There was no evidence of an increased risk of EBV-negative Hodgkin's lymphoma after infectious mononucleosis. In contrast, the risk of EBV-positive Hodgkin's lymphoma was significantly increased (relative risk, 4.0; 95 percent confidence interval, 3.4 to 4.5). The estimated median incubation time from mononucleosis to EBV-positive Hodgkin's lymphoma was 4.1 years (95 percent confidence interval, 1.8 to 8.3).

**CONCLUSIONS**

A causal association between infectious mononucleosis–related EBV infection and the EBV-positive subgroup of Hodgkin's lymphomas is likely in young adults.

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**D**ESPITE THE DISTINCTIVE EPIDEMIOLOGIC characteristics of Hodgkin's lymphoma, its cause is unknown.<sup>1</sup> In young adults, the disease may be a rare consequence of exposure to a common infectious agent.<sup>2</sup> There is, for example, a several-fold increase in the risk of Hodgkin's lymphoma after infectious mononucleosis, the typical clinical manifestation of primary Epstein-Barr virus (EBV) infection in adolescence.<sup>3</sup>

The suspicion that EBV has a causal role in Hodgkin's lymphoma is further strengthened by the demonstration of EBV antigens in Reed-Sternberg cells from 40 to 50 percent of patients with Hodgkin's lymphomas.<sup>3</sup> However, there is an apparent discrepancy<sup>4</sup>: the risk of Hodgkin's lymphoma after infectious mononucleosis is increased primarily among adolescents and young adults,<sup>5</sup> yet EBV can be demonstrated in Reed-Sternberg cells in only a third of patients with Hodgkin's lymphomas in that age group.<sup>6</sup>

This apparent inconsistency may be due to bias arising from misclassification caused by the similarity between the symptoms of Hodgkin's lymphoma and those of infectious mononucleosis. It is also possible that the connection between infectious mononucleosis-related EBV infection and Hodgkin's lymphoma in young adults is restricted to a subgroup of lymphomas that contain EBV, or that the virus is lost from Reed-Sternberg cells, the so-called hit-and-run hypothesis.<sup>7</sup>

In the present study, we examined these hypotheses using three population-based cohorts. We first assessed the risk of Hodgkin's lymphoma in two Danish cohorts of patients who had a Paul-Bunnell test for heterophil antibodies to determine whether they had infectious mononucleosis. A positive test was considered evidence of acute EBV infection and, hence, of EBV-related infectious mononucleosis, whereas a negative test was considered to indicate the absence of acute EBV infection and, hence, the absence of EBV-related infectious mononucleosis. We then characterized the EBV status of Hodgkin's lymphomas in patients who had had infectious mononucleosis. To increase the statistical power of our study, we combined the cohort of serologically positive Danish patients with a cohort of hospitalized Swedish patients who had infectious mononucleosis. All available biopsy specimens of Hodgkin's lymphomas that developed in these two cohorts of patients with infectious mononucleosis were tested for EBV antigens or EBV RNA. From these results,

we estimated the specific risks of EBV-positive and EBV-negative Hodgkin's lymphoma after the diagnosis of infectious mononucleosis.

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## METHODS

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### RISK OF HODGKIN'S LYMPHOMA AFTER A NEGATIVE OR POSITIVE PAUL-BUNNELL TEST

#### *Subjects*

In Denmark, the Statens Serum Institut served as the national reference laboratory for the serologic diagnosis of EBV infection between 1940 and 1978. The diagnostic method used was the Paul-Bunnell test for heterophil antibodies.<sup>8</sup> Information on name, dates of birth and testing, and test results was routinely recorded for all tested persons. The heterophil-positive cohort comprised all 22,017 persons with a positive Paul-Bunnell test (antibody titers of 1:32 or higher) between 1943 and 1978.<sup>9,10</sup> The heterophil-negative cohort comprised 32,790 persons who represented a random sample of persons who were tested for infectious mononucleosis and found to have a negative serologic result (antibody titers below 1:32) in the period from 1946 to 1978.<sup>10,11</sup>

#### *Linkages*

With information on date of birth, sex, and name, we used the Danish Civil Registration System to link the two cohorts and to determine each subject's unique 10-digit personal identification number (available since April 1, 1968) and vital status as of December 31, 1997. Personal identification numbers were established for 17,045 members of the seropositive cohort (77 percent) and for 24,614 members of the seronegative cohort (75 percent). Using the personal identification number as the key, we linked all subjects to the population-based Danish Cancer Registry in order to identify those with Hodgkin's lymphoma.

#### *Follow-up*

Follow-up for Hodgkin's lymphoma started on April 1, 1968, or the month after the Paul-Bunnell test, whichever came later, and ended on the date of diagnosis of Hodgkin's lymphoma, death, emigration, or December 31, 1997, whichever came first. The relative risk of Hodgkin's lymphoma after a Paul-Bunnell test was expressed as the standardized incidence ratio (i.e., the ratio of the observed number of cases of Hodgkin's lymphoma to the expected number). In each cohort, the expected numbers of Hodgkin's lymphomas were calculated with

use of the sex-, age-, and period-specific population-based incidence of Hodgkin's lymphoma and the corresponding cohort-specific person-years at risk. We estimated the relative risk of Hodgkin's lymphoma overall and according to the time since Paul-Bunnell testing, with 95 percent confidence intervals based on Wald's test, assuming that the observed cases followed a Poisson distribution.<sup>12</sup>

#### RISK OF EBV-POSITIVE AND EBV-NEGATIVE HODGKIN'S LYMPHOMA AFTER INFECTIOUS MONONUCLEOSIS

##### *Subjects, Linkages, and Follow-up*

To assess the risks of EBV-positive and EBV-negative Hodgkin's lymphoma after infectious mononucleosis with sufficient statistical power, we combined the Danish cohort of patients who had a positive Paul-Bunnell test with a Swedish cohort of 21,510 patients who had at one time received a diagnosis of infectious mononucleosis.<sup>5</sup> We identified all persons in the Swedish population-based Inpatient Registry who had received a discharge diagnosis of infectious mononucleosis between 1964 and 1994. Using the patient's unique national registration number, we linked this cohort to the Swedish Cause of Death Registry and the Registry of Population and Population Changes to ascertain the subjects' vital status as of 1995 and to the Swedish Cancer Registry to identify those with Hodgkin's lymphoma. Follow-up of the Swedish patients started the month after the diagnosis of infectious mononucleosis and ended on the date of diagnosis of Hodgkin's lymphoma, death, emigration, or December 31, 1995, whichever came first.

##### *Tumor Specimens*

Paraffin-embedded tumor specimens were available for 33 of the 46 patients with Hodgkin's lymphoma (72 percent) identified in the combined Danish-Swedish cohort of patients with infectious mononucleosis. After histologic validation of the diagnosis, there was sufficient material for EBV analyses for 32 of the 33 patients.

##### *EBV Analyses*

Antibodies for EBV analyses were obtained from Dako. Paraffin sections were stained with use of a standard immunohistochemical technique (EnVision, Dako). EBV latent membrane protein 1 was detected in Reed-Sternberg cells with antibody cocktail CS 1-4.<sup>13</sup> Antigen was retrieved by superheating

the samples in TEG buffer (10 nM TRIS and 0.5 nM EGTA, pH 9) in a microwave oven. EBV-encoded RNAs were detected by in situ hybridization with the use of single-stranded digoxigenin-labeled riboprobes.<sup>14</sup> In all samples that were negative for EBV-encoded RNA, in situ hybridization was repeated with use of a modified technique involving tyramine signal amplification.<sup>14</sup>

#### STATISTICAL ANALYSIS

Statistical analyses of the relation between infectious mononucleosis and the risk of EBV-positive and EBV-negative Hodgkin's lymphoma were restricted to the period two years or more after infectious mononucleosis in order to minimize bias in the follow-up of the cohort with a negative Paul-Bunnell test. This left 40 of the 46 original patients in the combined Danish-Swedish cohort. The EBV status of the tumor was established in 29 of the patients in whom Hodgkin's lymphomas developed during this follow-up period; the tumor EBV status in the remaining 11 patients was unknown.

Using odds ratios as a measure of relative risk, we first compared the distribution of EBV-positive and EBV-negative Hodgkin's lymphomas among the 29 patients with known EBV status with the distribution in an international, multicenter study that included 1105 patients with Hodgkin's lymphoma.<sup>6</sup> Taking a cohort approach, we assessed the overall relative risk (measured as standardized incidence ratios, as described above) of EBV-positive and EBV-negative Hodgkin's lymphoma after infectious mononucleosis. Since population-based incidence rates are available only for Hodgkin's lymphoma overall, we approximated EBV-status-specific rates by apportioning age-, sex-, period-, and country-specific incidence rates for EBV-positive and EBV-negative Hodgkin's lymphoma according to distributions identified on direct testing of EBV tumors.<sup>6</sup>

We next characterized the temporal variation in the risk of EBV-positive and EBV-negative Hodgkin's lymphoma after infectious mononucleosis and estimated the median incubation period of infectious mononucleosis-related Hodgkin's lymphoma. Specifically, in a series of analyses we tested the assumption that the relative risk of EBV-positive and EBV-negative Hodgkin's lymphoma, respectively, was constant during the interval after infectious mononucleosis against the alternative assumption that the relative risk varied over time owing to an ad-

ditional risk of lymphoma that was directly attributable to infectious mononucleosis and that was assumed to follow the bell-shaped pattern characteristic of incubation-period distributions of several infectious diseases. We performed three of these analyses, each of which included the 11 patients with Hodgkin's lymphoma whose tumor EBV status was unknown: one analysis assumed that all 11 patients were EBV-positive, one assumed they were all EBV-negative, and one (the most likely scenario) assumed that the missing data on viral status were uninformative with respect to the true EBV status. The technical details of the statistical analyses are provided in the Supplementary Appendix, available with the full text of this article at <http://www.nejm.org>.

RESULTS

**RISK OF HODGKIN'S LYMPHOMA AFTER A NEGATIVE OR POSITIVE PAUL-BUNNELL TEST**

Demographic characteristics of the Danish patients with a positive Paul-Bunnell test for heterophil antibodies and the patients with a negative test are shown in Table 1, together with similar information on the cohort of Swedish patients with infectious mononucleosis. In the cohort with a negative Paul-Bunnell test, the relative risk of Hodgkin's lymphoma was increased during the first two years of follow-up but not thereafter (Table 2). In the cohort with a positive Paul-Bunnell test, by contrast, the relative risk was more than doubled for up to two decades after testing (Table 2). These two patterns of relative risk were significantly different (P=0.004).

**Table 1. Person-Years and Subjects at Risk for Hodgkin's Lymphoma in the Danish Cohort of Subjects with Positive or Negative Results on the Paul-Bunnell Test and in the Swedish Cohort of Patients with Infectious Mononucleosis, According to Sex and the Time since the Diagnosis of Infectious Mononucleosis.**

Variable	Danish Cohort		Swedish Cohort
	Negative Paul-Bunnell Test	Positive Paul-Bunnell Test	
	no. of person-years (no. of subjects)		
Sex			
Female	281,542 (12,041)	203,929 (7702)	121,523 (9673)
Male	296,720 (12,573)	242,827 (9343)	151,083 (11,837)
Time since diagnosis			
<1 Yr	12,937 (14,500)	6,504 (7208)	20,555 (21,510)
1 Yr	14,245 (14,800)	7,664 (8144)	20,919 (21,453)
2-4 Yr	47,139 (16,887)	27,845 (10,324)	57,143 (20,461)
5-9 Yr	87,493 (18,977)	56,306 (12,300)	75,723 (17,661)
10-14 Yr	95,914 (20,465)	64,694 (13,881)	50,619 (12,595)
15-19 Yr	99,167 (21,584)	71,505 (15,312)	29,406 (7902)
≥20 Yr	221,367 (16,592)	212,239 (15,602)	18,241 (4114)
Total	578,262 (24,614)	446,757 (17,045)	272,606 (21,510)

**RISK OF EBV-POSITIVE AND EBV-NEGATIVE HODGKIN'S LYMPHOMA AFTER INFECTIOUS MONONUCLEOSIS**

The diagnosis of Hodgkin's lymphoma was confirmed in the 33 biopsy specimens from the 46 patients in the combined Danish and Swedish cohorts with infectious mononucleosis (21 and 25 patients, respectively). The main characteristics of

**Table 2. Observed and Expected Numbers of Cases of Hodgkin's Lymphoma and Relative Risk of Hodgkin's Lymphoma in the Danish Cohort, According to the Results on the Paul-Bunnell Test, and in the Swedish Cohort of Patients with Infectious Mononucleosis.\***

Variable	Danish Cohort						Swedish Cohort		
	Negative Paul-Bunnell Test			Positive Paul-Bunnell Test			Observed	Expected	RR (95% CI)
	Observed	Expected	RR (95% CI)	Observed	Expected	RR (95% CI)			
Overall	55	16.35	3.3 (2.6-4.4)	21	12.72	1.7 (1.1-2.5)	25	6.17	4.1 (2.7-6.0)
Time since diagnosis									
<1 Yr	31	0.32	95.9 (67.4-136.4)	0	0.15	—	4	0.36	11.0 (4.1-29.2)
1 Yr	5	0.37	13.5 (5.6-32.5)	1	0.19	5.1 (0.7-36.5)	1	0.40	2.5 (0.4-17.8)
2-4 Yr	1	1.29	0.8 (0.1-5.5)	3	0.82	3.7 (1.2-11.3)	8	1.21	6.6 (3.3-13.2)
5-9 Yr	5	2.52	2.0 (0.8-4.8)	6	1.86	3.2 (1.4-7.2)	7	1.85	3.8 (1.8-8.0)
10-19 Yr	6	5.73	1.0 (0.5-2.3)	9	4.18	2.2 (1.1-4.1)	5	1.98	2.5 (1.0-6.1)
≥20 Yr	7	6.12	1.1 (0.5-2.4)	2	5.51	0.4 (0.1-1.5)	0	0.36	—

\* RR denotes relative risk, and CI confidence interval.

**Table 3. Characteristics of 46 Swedish and Danish Patients in Whom Hodgkin's Lymphoma Developed after Infectious Mononucleosis.\***

Patient No.	Country	Sex	Interval between Diagnoses	Hodgkin's Lymphoma				
				Subtype	Age at Diagnosis yr	LMP-1 Status	EBER Status	EBV Status
1	Sweden	Male	1 mo	Nodular sclerosis	28	-	-	-
2	Sweden	Male	6 mo	Tumor not available	19			
3	Sweden	Female	7 mo	Tumor not available	22			
4	Sweden	Male	11 mo	Lymphocytic predominance	52	-	-	-
5	Denmark	Male	12 mo	Mixed cellularity	24	+	+	+
6	Sweden	Male	1 yr 10 mo	Tumor not available	19			
7	Sweden	Male	2 yr 4 mo	Mixed cellularity	25	+	+	+
8	Denmark	Male	2 yr 4 mo	Tumor not available	29			
9	Sweden	Female	2 yr 5 mo	Tumor not available	24			
10	Sweden	Female	2 yr 7 mo	Tumor not available	19			
11	Sweden	Male	2 yr 9 mo	Nodular sclerosis	19	+	+	+
12	Denmark	Male	2 yr 10 mo	Mixed cellularity	24	+	+	+
13	Sweden	Male	2 yr 10 mo	Mixed cellularity	28	+	+	+
14	Sweden	Male	3 yr	Mixed cellularity	7	+	+	+
15	Sweden	Male	3 yr	Nodular sclerosis	30	+	+	+
16	Sweden	Male	4 yr 10 mo	Mixed cellularity	26	+	+	+
17	Denmark	Male	4 yr 11 mo	Tumor not available	23			
18	Sweden	Female	5 yr 7 mo	Nodular sclerosis	22	NS	+	+
19	Sweden	Male	6 yr 2 mo	Mixed cellularity	15	+	+	+
20	Denmark	Female	6 yr 4 mo	Tumor not available	23			
21	Sweden	Female	6 yr 11 mo	Nodular sclerosis	21	-	-	-
22	Denmark	Female	7 yr	Tumor not available	22			
23	Sweden	Male	7 yr 5 mo	Mixed cellularity	30	NS	+	+
24	Sweden	Female	7 yr 9 mo	Nodular sclerosis	28	+	+	+
25	Denmark	Male	8 yr 5 mo	Mixed cellularity	34	+	+	+
26	Sweden	Female	9 yr	Nodular sclerosis	25	-	-	-
27	Denmark	Male	9 yr 1 mo	Nodular sclerosis	27	+	+	+
28	Denmark	Male	9 yr 2 mo	Mixed cellularity	28	+	+	+
29	Denmark	Male	9 yr 5 mo	Nodular sclerosis	31	-	-	-
30	Sweden	Female	9 yr 9 mo	Nodular sclerosis	27	-	-	-
31	Sweden	Male	10 yr	Nodular sclerosis	30	-	-	-
32	Denmark	Male	10 yr 8 mo	Tumor not available	26			
33	Denmark	Female	12 yr 3 mo	Nodular sclerosis	27	-	-	-
34	Sweden	Male	12 yr 7 mo	Nodular sclerosis	19	-	-	-
35	Denmark	Female	12 yr 8 mo	Nodular sclerosis	29	+	+	+
36	Sweden	Male	13 yr 9 mo	Tumor not available	19			
37	Sweden	Male	14 yr 3 mo	Nodular sclerosis	33	-	-	-
38	Denmark	Male	14 yr 9 mo	Nodular sclerosis	32	+	+	+
39	Denmark	Female	15 yr 2 mo	Tumor not available	32			
40	Sweden	Male	15 yr 2 mo	Nodular sclerosis	73			

Table 3. (Continued.)

Patient No.	Country	Sex	Interval between Diagnoses	Hodgkin's Lymphoma				
				Subtype	Age at Diagnosis yr	LMP-1 Status	EBER Status	EBV Status
41	Denmark	Male	16 yr 3 mo	Lymphocytic predominance	39	–	–	–
42	Denmark	Male	16 yr 11 mo	Nodular sclerosis	39	–	–	–
43	Denmark	Male	17 yr 4 mo	Nodular sclerosis	37	–	–	–
44	Denmark	Male	19 yr 11 mo	Nodular sclerosis	34	–	–	–
45	Denmark	Female	27 yr 9 mo	Tumor not available	65			
46	Denmark	Male	38 yr 7 mo	Lymphocytic predominance	57	–	–	–

\* Biopsy specimens from 33 patients were available for histopathological analysis. In the case of Patient 40, there was insufficient material for immunohistochemical staining of Reed–Sternberg cells for Epstein–Barr virus (EBV) latent membrane protein (LMP-1) or in situ hybridization of Reed–Sternberg cells for EBV-related RNA (EBER). NS denotes an analysis that was not successful. Minus signs indicate negative results, and plus signs positive results.

the 46 patients are given in Table 3. Among the 33 specimens, 20 (61 percent) were the nodular sclerosis subtype of Hodgkin's lymphoma, 10 (30 percent) were the mixed cellularity subtype, and 3 (9 percent) were the lymphocytic predominance subtype. EBV was demonstrated in 17 of the 32 specimens that were tested (53 percent). The proportion of EBV-positive specimens was similar among the Danish patients (7 of 14 tumors) and the Swedish patients (10 of 18) and among male patients (14 of 25) and female patients (3 of 7). Seven of 19 specimens with nodular sclerosis were positive for EBV, as were all 10 specimens with mixed cellularity; in contrast, none of the 3 specimens with lymphocytic predominance were EBV-positive.

In statistical analyses restricted to the 29 tumors with known EBV status that were diagnosed more than two years after infectious mononucleosis occurred, EBV-positive tumors constituted a larger proportion (55 percent) than expected (31 percent), on the basis of a previous study of Hodgkin's lymphoma<sup>6</sup> (odds ratio, 2.7; 95 percent confidence interval, 1.2 to 6.0). Using estimated incidence rates of EBV-status-specific Hodgkin's lymphoma, we found that the relative risk of EBV-positive Hodgkin's lymphoma was 2.8 (95 percent confidence interval, 1.7 to 4.6), which was significantly higher than the relative risk of EBV-negative Hodgkin's lymphoma (1.1 [95 percent confidence interval, 0.7 to 2.0],  $P=0.015$ ).

Figure 1 shows the relative risks of EBV-positive

and EBV-negative Hodgkin's lymphoma according to three statistical models that included all 40 observed cases of lymphoma. In all three scenarios (all 11 patients whose tumor EBV status was unknown were assumed to be EBV-positive, all were assumed to be EBV-negative, or missing data on viral status were assumed to be uninformative with respect to the true EBV status), the distribution of EBV-negative cases of Hodgkin's lymphoma was consistent with the existence of a constant relative risk rather than a bell-shaped relative risk (smallest  $P$  value = 0.74). In contrast, the distribution of EBV-positive cases of Hodgkin's lymphoma was inconsistent with the existence of a constant relative risk rather than a bell-shaped pattern of risk in all three scenarios (largest  $P$  value < 0.001).

In the model in which the distribution of EBV-positive and EBV-negative cases among the 11 patients with untested tumors was assumed to be similar to that of the 29 tested lymphomas, the relative risk of EBV-negative Hodgkin's lymphoma was 1.5 overall (95 percent confidence interval, 0.9 to 2.5), and the relative risk of EBV-positive Hodgkin's lymphoma was 4.0 overall (95 percent confidence interval, 3.4 to 4.5), but it varied considerably over time (Fig. 1). The estimated median incubation period for EBV-positive Hodgkin's lymphoma attributable to infectious mononucleosis was 4.1 years (95 percent confidence interval, 1.8 to 8.3), whereas the risk peaked after 2.4 years (95 percent confidence interval, 1.1 to 5.0).

## DISCUSSION

We investigated the risk of Hodgkin's lymphoma among persons with a history of infectious mononucleosis and found that the risk of EBV-positive tumors was increased among such persons, whereas the risk of EBV-negative tumors did not differ from the expected risk. These observations indicate a causal association between infectious mononucleosis-related EBV infection and EBV-positive Hodgkin's lymphoma in young adults.

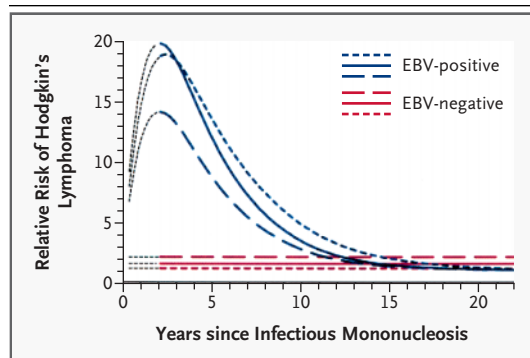
Several studies have reported that there is an increased risk of Hodgkin's lymphoma shortly after infectious mononucleosis is diagnosed.<sup>3,5</sup> Although an immediate increase in the risk may reflect rapid progression from EBV infection to Hodgkin's lymphoma, it is more likely due to the overlapping clinical presentations of infectious mononucleosis and Hodgkin's lymphoma. We investigated this possibility by comparing the risk of Hodgkin's lymphoma in two cohorts in which infectious mononucleosis was suspected clinically. One cohort was seropositive, and the other was seronegative. The seronegative cohort had a marked and immediate increased

risk of Hodgkin's lymphoma, which dropped to unity within the first two years of follow-up. In contrast, the risk of Hodgkin's lymphoma in the seropositive cohort remained increased for two decades. Some members of the seronegative cohort may have had false negative Paul-Bunnell tests, and infectious mononucleosis-related Hodgkin's lymphoma may have developed in these subjects. It is more likely, however, that the transient increase in risk in the seronegative cohort reflects the inclusion of patients with mononucleosis-like symptoms that were actually caused by incipient Hodgkin's lymphoma.

There may have been similar biases with respect to the combined cohort of Danish patients with positive Paul-Bunnell tests and Swedish patients who were hospitalized with infectious mononucleosis. Together, these findings indicate that the increased risk of Hodgkin's lymphoma observed shortly after infectious mononucleosis may be partly explained by bias and factors that are unrelated to the EBV infection. In contrast, these biases cannot explain our finding of a long-lasting increased risk of Hodgkin's lymphoma after infectious mononucleosis.

As a second step in our study, we determined whether EBV antigens and EBV RNA were present in the biopsy specimens of Hodgkin's lymphoma from the combined Danish-Swedish cohort of patients who had a history of infectious mononucleosis. These analyses revealed a higher-than-expected proportion of EBV-positive tumors, with an estimated odds ratio for EBV-positive Hodgkin's lymphoma of 2.7 (95 percent confidence interval, 1.2 to 6.0). We know of only two other studies that have analyzed the EBV status of patients with Hodgkin's lymphoma in relation to a history of infectious mononucleosis, and they yielded conflicting results.<sup>15,16</sup> Unlike previous investigators,<sup>15,16</sup> we were able to assess not only the internal distribution of EBV-positive and EBV-negative Hodgkin's lymphoma but also the risks of EBV-positive and EBV-negative Hodgkin's lymphoma, relative to those expected in the general population and according to the time since the diagnosis of infectious mononucleosis. These analyses showed an increased and bell-shaped pattern of the relative risk of EBV-positive Hodgkin's lymphoma after infectious mononucleosis, whereas there was no evidence of a similar pattern for the relative risk of EBV-negative Hodgkin's lymphoma. Indeed, we found little to suggest an increased risk of EBV-negative Hodgkin's lymphoma overall.

Additional statistical analyses indicated that bias



**Figure 1. Relative Risk of Epstein-Barr Virus (EBV)-Positive and EBV-Negative Hodgkin's Lymphoma after Infectious Mononucleosis.**

Solid lines represent the relative risks of EBV-positive (blue) and EBV-negative (red) Hodgkin's lymphoma, given that EBV status was determined in an unbiased way and that the missing data on viral status in 11 tumors were uninformative with respect to their true EBV status. Short-dashed lines represent the relative risks of EBV-positive and EBV-negative Hodgkin's lymphoma given that all tumors whose EBV status was unknown were EBV-positive. Long-dashed lines represent the relative risks given that all tumors whose EBV status was unknown were EBV-negative. The analyses were restricted to the period two years or more after infectious mononucleosis.

in the retrieval of EBV-positive and EBV-negative tumors for viral analyses could not explain the observed time-dependent variation in the incidence of EBV-positive Hodgkin's lymphoma after infectious mononucleosis. The finding that the risk of EBV-negative tumors was not increased suggests that this subgroup of Hodgkin's lymphoma is not associated with infectious mononucleosis-related EBV infection, thus challenging the proposed hit-and-run mechanism of viral escape in young-adult Hodgkin's lymphoma.<sup>7</sup> Rather, our results indicate that EBV infection in the form of infectious mononucleosis is causally associated with EBV-positive Hodgkin's lymphoma in young adults.

We estimated that the median incubation period for Hodgkin's lymphoma attributable to infectious mononucleosis-related EBV infection was 4.1 years, with a peak in risk 2.4 years after infection. These estimates are in accordance with those of a large case-control study of Hodgkin's lymphoma in which the median interval between infectious mononucleosis and Hodgkin's lymphoma was five years.<sup>17</sup>

In contrast to previous studies of the association between infectious mononucleosis and EBV-positive Hodgkin's lymphoma,<sup>15,16</sup> ours did not rely on a self-reported history of mononucleosis. Rather, information on mononucleosis and Hodgkin's lymphoma was identified in independently and prospectively collected population-based data, and tumor analyses were carried out in our laboratory, thereby excluding recall bias.

We used published data on the proportion of EBV-positive and EBV-negative Hodgkin's lymphomas in different age groups<sup>6</sup> to estimate the EBV-status-specific relative risks of Hodgkin's lymphoma. Although we cannot be sure that the estimated rates correspond exactly to the proportion of EBV-positive lymphomas in Sweden and Denmark, the

use of other previously reported but smaller Danish<sup>18</sup> and Swedish<sup>19</sup> studies of the prevalence of EBV in Hodgkin's lymphoma did not materially alter our risk estimates (data not shown).

We were able to assess the EBV status of 32 of the 46 patients with Hodgkin's lymphoma. Theoretically, we cannot rule out the possibility of a marginally increased risk of EBV-negative Hodgkin's lymphoma (though not a bell-shaped pattern) in the event that a disproportionate percentage of patients whose tumor EBV status was unknown were EBV-negative. However, we believe that bias in the retrieval of tumors for EBV testing was unlikely to have occurred.

EBV was detected by highly sensitive methods; in situ hybridization can detect single EBV-encoded RNA particles in archival material.<sup>20</sup> The histologic subtypes in the analyzed tumor samples corresponded closely with the expected distribution among young adults, and the uneven distribution of EBV-positive and EBV-negative tumors among the histologic subtypes was consistent with previous data.<sup>6</sup> However, since our patients were predominantly young adults, we cannot draw conclusions about associations between infectious mononucleosis-related EBV infection and EBV-positive Hodgkin's lymphoma in either children or elderly patients or about Hodgkin's lymphomas that occur after subclinical EBV infection.

We emphasize that in absolute terms, the risk of Hodgkin's lymphoma after infectious mononucleosis is only on the order of 1 case per 1000 persons.<sup>5</sup> Consequently, other cofactors acting in concert with infectious mononucleosis-related EBV infection presumably must be present for the infection to give rise to Hodgkin's lymphoma.<sup>16</sup>

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