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Borrelia infection and risk of non-Hodgkin lymphoma

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Reports of the presence of *Borrelia burgdorferi* DNA in malignant lymphomas have raised the hypothesis that infection with *B burgdorferi* may be causally related to non-Hodgkin lymphoma (NHL) development. We conducted a Danish-Swedish case-control study including 3055 NHL patients and 3187 population controls. History of tick bite or *Borrelia* infection was ascertained through structured telephone interviews and through enzyme-linked immunosorbent assay serum analyses for antibodies against *B burgdorferi* in a subset of 1579 patients and 1358 controls. Statistical associations with risk of NHL, including histologic subtypes, were assessed by logistic regression. Overall risk of NHL was not associated with self-reported history of tick bite (odds ratio [OR] = 1.0; 95% confidence interval: 0.9-1.1), *Borrelia* infection (OR = 1.3 [0.96-1.8]) or the presence of anti-*Borrelia* antibodies (OR = 1.3 [0.9-2.0]). However, in analyses of NHL subtypes, self-reported history of *B burgdorferi* infection (OR = 2.5 [1.2-5.1]) and seropositivity for anti-*Borrelia* antibodies (OR = 3.6 [1.8-7.4]) were both associated with risk of mantle cell lymphoma. Notably, this specific association was also observed in persons who did not recall *Borrelia* infection yet tested positive for anti-*Borrelia* antibodies (OR = 4.2 [2.0-8.9]). Our observations suggest a previously unreported association between *B burgdorferi* infection and risk of mantle cell lymphoma. (Blood. 2008;111: 5524-5529)

Introduction

In recent years, a growing number of infectious agents have been linked to non-Hodgkin lymphoma (NHL), including, for example, *Chlamydia psittaci,* hepatitis C virus, *Campylobacter jejuni,* and *Helicobacter pylori*.[1-4] While each of these infectious agents accounts only for a small proportion of the total number of NHL cases, the observed associations are important because they have clinical and therapeutic implications and provide novel insight into the mechanisms that govern lymphoma development.

Patients with symptomatic infection with the spirochete *Borrelia burgdorferi* display characteristic manifestations that may include skin rash, arthritis, and neurologic deficits, a clinical picture commonly referred to as Lyme disease or borreliosis.[5] In some cases, *B burgdorferi* infection may also entail chronic inflammation of the skin with dense lymphocytic infiltration followed by atrophy, known as acrodermatitis chronica atrophicans (ACA).[6] This chronic inflammatory state of the skin resembles the setting in which chronic *Helicobacter pylori* infection may induce mucosa-associated lymphoid tissue (MALT) lymphomas in the stomach.[7] Interestingly, several cases of cutaneous B-cell lymphomas have been reported to develop in the context of ACA.[8]

The suspicion of a causal association between *B burgdorferi* and cutaneous lymphomas has gained further credibility by serologic evidence of *B burgdorferi* infection in lymphoma patients,[9,10] and in particular by the demonstration of *B burgdorferi* DNA in a proportion of lymphoma skin lesions.[11-13] Moreover, regression of lymphomas upon treatment of the *Borrelia* infection has also been reported.[13,14]

While the evidence that *B burgdorferi* may be implicated in development of cutaneous B-cell lymphomas is considerable, the question remains if the association may also pertain to noncutaneous lymphomas. Infection with *B burgdorferi* is indeed not limited to the skin, but, as reflected by its wide range of clinical manifestations, also disseminates to other regions including, presumably, the lymphoid tissues.[15] It is, therefore, of interest that we recently demonstrated the presence of *B burgdorferi* DNA within the malignant lesions of 2 patients with nodal B-cell lymphoma.[16]

Inspired by this body of evidence, we investigated the hypothesis that *B burgdorferi* infection is associated with an increased risk of NHL overall or specific subtypes of NHL, reflecting correspondingly increased risks of cutaneous and—to a lesser extent—noncutaneous lymphomas.

Methods

Study population

The study was based on data and biologic materials collected in a nationwide Danish-Swedish case-control study (Scandinavian Lymphoma Etiology study or SCALE) from 1999 to 2002 as previously described.[17] It encompassed residents 18 to 74 years old, living in either Denmark from June 1, 2000, to August 30, 2002, or Sweden from October 1, 1999, to April

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15, 2002. Participants in a Danish regional pilot study that began November 1, 1999, and gradually expanded to cover the entire country were also included. Eligible cases in SCALE were those with a primary, incident, and morphologically verified diagnosis of NHL. Participants were required to speak Danish or Swedish and to have no history of organ transplantation, HIV infection, or prior hematopoietic malignancy. Case patients were identified through a rapid case ascertainment network including all hospital departments where malignant lymphomas are diagnosed and treated in Denmark and Sweden. Continuous collaboration with the 6 regional cancer registries in Sweden and the Danish National Pathology Registry ensured complete reporting through the network. Controls were randomly sampled from the entire Danish and Swedish populations using continuously updated, computerized population registers. Thus, a subset of controls was sampled every 6 months during the study period, frequency-matched within each country to the expected distribution of cases of NHL, by sex and age (in 10-year intervals).

The study was approved by regional ethics committees in both countries. Informed consent was obtained from each participant before interview and blood sampling in accordance with the Declaration of Helsinki.

Histopathologic classification

In Denmark, review of tumor material from cases was performed within the Danish Lymphoma Group Registry (LYFO),[18] where 10% of all incident cases in the country are continuously randomly chosen and reviewed by expert hematopathologists. In addition, LYFO-approved senior hematopathologists performed the primary evaluation of the diagnostic tumor specimens of all but 20% of the study cases.[17]

In Sweden, all cases were histopathologically evaluated by 1 of 6 senior hematopathologists/cytologists affiliated with the study. The original diagnostic tumor slides were reviewed for all but 1.5% of cases, for whom the written results of the primary morphologic and immunohistochemical investigation were evaluated.[17]

All cases in both countries were classified according to the current World Health Organization classification of hematopoietic and lymphoid tumors.[19] Information on lymphoma topography and location (nodal/extranodal) was obtained through LYFO in Denmark and 6 regional lymphoma registries in Sweden.

Exposure assessment

Exposure to *B burgdorferi* infection was assessed in 2 ways. First, all study participants completed a structured telephone interview with questions addressing a wide range of potential risk factors for malignant lymphoma, including history of tick bites and Lyme disease (yes/no; if yes, at which age). Second, serum samples were provided by 85% of the enrolled patients and 65% of the enrolled controls.

We evaluated questionnaire data on self-reported *Borrelia* infection from all SCALE participants (3055 patients with NHL and 3187 control persons). In addition, we analyzed sera from a subset of participants for anti–*B burgdorferi* IgG antibodies. The serologic analyses included all participants with self-reported *Borrelia* infection, cases whose blood sample was before initiation of lymphoma treatment or where the timing was uncertain, and a random two thirds of the controls. Thus, the serologic analyses included 1579 NHL patients and 1358 controls. The serologic analyses were carried out at Statens Serum Institute using an in-house accredited enzyme-linked immunosorbent assay (ELISA); each ELISA plate including standard sera with known titers to allow appropriate adjustments for day-to-day and plate-to-plate variation (Danish Accreditation and Metrology Fund [DANAK, Skovlunde, Denmark] according to ISO 17025).

Statistical analyses

The association between infection with *B burgdorferi* and risk of NHL, overall and by subtypes, was evaluated using unconditional logistic regression. Odds ratios (ORs) and 95% confidence intervals (CIs), as measures of relative risk, were first calculated with adjustment for the matching variables (age in 10-year intervals, country of residence, and sex). Additional adjustment for factors known or suspected as potential risk factors of NHL[20] and possibly associated with *Borrelia* infection, including education level (≤ 9 years, 10-12 years, > 12 years), outdoor occupation lasting 1 year or more (ever/never), farming and forest working (ever/never), occupation involving exposure to organic solvents (yes/no) or pesticides (yes/no), daily contact with pets after the age of 15 years lasting 1 year or more (ever/never), and a medical history of skin cancer, autoimmune disease, or allergy, changed estimates only marginally and were therefore not included in the final model. The likelihood ratio test was used to test for statistical significance of independent variables and interaction effects. *P* values less than 5% were considered statistically significant. All statistical tests were 2-sided.

Results

Overall, 3055 patients (81% of all identified incident cases) with NHL and 3187 controls (71% of those contacted) were enrolled in the SCALE study. Table 1 shows the distribution of participants by age, sex, country of residence, and self-reported history of tick bite or *Borrelia* infection for NHL overall and for NHL subtypes with more than 100 cases. Table 1 also summarizes the results of the serologic analyses for anti-*Borrelia* antibodies. Six participants (4 controls and 2 NHL cases) had serologic values interpreted as intermediate, and were therefore excluded from statistical analyses of the serologic results.

Tick bite history was not associated with risk of NHL overall or specific NHL subtypes (Table 2). Self-reported history of *Borrelia* infection was not associated with an increased risk of NHL overall (OR = 1.3; 95% CI: 0.96-1.8). In analyses stratified by histologic subtype, however, self-reported *Borrelia* infection was associated with an elevated risk of mantle cell lymphoma (MCL) (OR = 2.5; 95% CI: 1.2-5.1; Table 2).

These findings were borne out by the serologic analyses. Thus, serologic evidence of *Borrelia* infection was not associated with the risk of all types of NHL combined (OR = 1.3; 95% CI: 0.9-2.0), whereas an increased risk was seen for MCL (OR = 3.6; 95% CI: 1.8-7.4; Table 2). Similar risk estimates were obtained in analyses including the persons with intermediate serologic results as seropositive or seronegative (data not shown). In supplementary analyses stratified according to the combination of self-reported and serologic data, the increased risk of MCL was observed both in seronegative persons with self-reported *Borrelia* infection (OR = 3.1; 95% CI: 1.3-7.4) and in seropositive persons without self-reported history of *Borrelia* infection (OR = 4.2; 95% CI: 2.0-8.9), whereas neither the risk of all subtypes of NHL combined nor of other individual lymphoma subtypes was increased in these strata (data not shown). Among persons with both self-reported and serologic evidence of *Borrelia* infection, elevated risk estimates were observed for virtually all investigated lymphoma subtypes and consequently for all NHL subtypes combined (OR = 3.5; 95% CI: 1.3-9.4). The total number of exposed controls contributing to these analyses was, however, small (n = 5), and we therefore suspect that the universally increased lymphoma risk in the latter analysis reflects a spurious distribution among the controls.

Among tested controls, more men (31/699 = 4%) than women (13/611 = 2%) and more Swedes (34/823 = 4%) than Danes (10/531 = 2%) were seropositive, but the seroprevalence did not vary by age or educational level (data not shown). The higher seropositivity in males and in Sweden mirrored a similarly skewed distribution of self-reported history of tick bite by sex and country (data not shown).

The observed association between risk of NHLs overall or NHL subtypes and *Borrelia* seropositivity did not vary by timing of

Table 1. General and disease characteristics of controls and patients with non-Hodgkin lymphoma (NHL) overall and NHL subtypes

Characteristic/disease	Controls, no. (%)*	All NHLs, no. (%)*	DLBCL, no. (%)*	CLL, no. (%)*	FL, no. (%)*	NHL subtypes MCL, no. (%)*	MZL, no. (%)*	LPL, no. (%)*	TCL, no. (%)*
SCALE participants overall	3187 (100)	3055 (100)	796 (100)	752 (100)	586 (100)	148 (100)	117 (100)	116 (100)	204 (100)
Age, y									
18 to 44	576 (18)	334 (10)	131 (16)	21 (3)	73 (12)	4 (3)	8 (7)	4 (4)	57 (28)
45 to 54	597 (19)	587 (19)	143 (18)	138 (18)	141 (24)	26 (18)	22 (19)	16 (14)	38 (19)
55 to 64	906 (28)	1011 (33)	247 (31)	257 (34)	206 (35)	52 (35)	41 (35)	48 (41)	54 (26)
65 to 74	1108 (35)	1123 (37)	275 (35)	336 (45)	166 (28)	66 (45)	46 (39)	48 (41)	55 (27)
Median (range)	59 (18-76)	60 (18-74)	60 (19-74)	63 (30-74)	57·5 (22-74)	63 (34-74)	62 (26-74)	62 (28-74)	55 (18-74)
Sex									
Male	1767 (55)	1819 (60)	474 (60)	480 (64)	279 (48)	112 (76)	57 (49)	75 (65)	128 (63)
Female	1420 (45)	1236 (40)	322 (40)	272 (36)	307 (52)	36 (24)	60 (51)	41 (35)	76 (37)
Country of residence									
Denmark	1186 (37)	1075 (35)	283 (36)	296 (39)	222 (38)	54 (36)	49 (42)	50 (43)	77 (38)
Sweden	2001 (63)	1980 (65)	513 (64)	456 (61)	364 (62)	94 (64)	68 (58)	66 (57)	127 (62)
Tick bite (self-reported)									
No	1853 (59)	1736 (58)	454 (59)	422 (57)	355 (61)	81 (55)	68 (60)	63 (55)	127 (64)
Yes	1263 (41)	1248 (42)	319 (41)	318 (43)	224 (39)	65 (45)	46 (40)	51 (45)	71 (36)
***Borrelia* infection (self-reported)**									
No	3082 (98)	2928 (97)	766 (97)	722 (97)	561 (97)	138 (94)	117 (100)	111 (96)	194 (96)
Yes	78 (2)	102 (3)	26 (3)	23 (3)	19 (3)	9 (6)	—	5 (4)	8 (4)
Time since *Borrelia* infection									
Less than 1 y	9 (12)	13 (13)	4 (16)	4 (17)	2 (11)	—	—	—	1 (11)
1 to 4 y	30 (39)	44 (44)	9 (36)	9 (39)	12 (63)	4 (50)	—	1 (20)	5 (56)
5 to 9 y	20 (26)	18 (18)	4 (16)	7 (30)	2 (11)	2 (25)	—	1 (20)	1 (11)
10 to 14 y	11 (14)	12 (12)	3 (12)	2 (9)	2 (11)	2 (25)	—	—	1 (11)
More than 15 y	6 (8)	14 (14)	5 (20)	1 (4)	1 (5)	—	—	3 (60)	1 (11)
Anti-*Borrelia* antibodies†									
Negative	1310 (97)	1504 (95)	332 (95)	563 (96)	347 (96)	74 (86)	66 (97)	4 (100)	111 (97)
Positive	44 (3)	73 (5)	18 (5)	23 (4)	13 (4)	12 (14)	2 (3)	—	3 (3)

DLBCL indicates diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; LPL, lymphoplasmacytic lymphoma; and TCL, T-cell lymphoma.

*Numbers may not add up due to missing information or minor NHL subtypes not included in the subtype analyses.

†*Borrelia*-specific IgG antibodies were analyzed in serum samples of 1579 cases with NHL and 1358 matched controls; in 6 participants (2 cases and 4 controls) serologic analyses yielded inconclusive results and were excluded.

blood sampling relative to treatment (before or unknown) among the cases (data not shown).

Information about tumor topography was available for 65 of the 73 *Borrelia*-seropositive NHL patients. Sixteen patients (16/65 = 25%) were registered with extranodal or combined nodal-extranodal disease, of which 4 (4/65 = 6%) were located in the skin (1 case each of chronic lymphocytic leukemia [CLL], follicular lymphoma [FL], MCL, and T-cell lymphoma). Other extranodal locations were stomach (3 diffuse large B-cell lymphomas [DLBCLs], 1 marginal zone lymphoma [MZL]), subcutis (1 DLBCL), bone (1 DLBCL), colon (1 MCL), liver (1 DLBCL), muscle (1 T-cell lymphoma), salivary glands (1 DLBCL, 1 MZL), and sinus (1 DLBCL). In seronegative NHL patients, 307 of 1242 with available data had an extranodal or combined nodal-extranodal localized tumor (25%), of which 39 patients (11 DLBCLs, 9 FLs, 1 MCL, 2 MZLs, 16 T-cell lymphomas) were registered with a cutaneous lymphoma (39/1242 = 3%). However, although twice as frequent in *Borrelia*-infected patients, the distribution of neither cutaneous lymphomas ($P = .25$) nor lymphoma with other extranodal locations differed statistically between *Borrelia*-seropositive and seronegative NHL patients (data not shown).

Discussion

In this large, population-based case-control study, history of *Borrelia* infection was associated with a nearly 3-fold increased risk of MCL, whether the exposure was self-reported or based on serologic evidence, even in patients with no recollection of *Borrelia* disease.

An association between *Borrelia* infection and lymphoma development has been suspected since even before the identification of the spirochete in 1982.[21] Accordingly, the occurrence of lymphoma in close proximity to typical skin manifestations of borreliosis, such as ACA and lymphadenosis benigna cutis, has been described in several case reports.[22,23] However, other and more tangible evidence of a causal association in certain lymphoma subtypes includes elevated titers of anti-*Borrelia* antibodies described in individual patients with primary cutaneous B-cell lymphomas (PCBCL),[8,9] a higher anti-*Borrelia* antibody seroprevalence among PCBCL patients than among controls,[10] cultivation of the spirochete from skin lesions of 2 PCBCL patients,[13] and demonstration of *Borrelia* DNA in cutaneous B-cell[11,12] and T-cell lymphoma lesions[24] by polymerase chain reaction (PCR) techniques. Until recently, there has been little evidence of a *Borrelia*-lymphoma association in studies outside Europe,[25-27] which has been attributed to variations in the clinical manifestations of infection with different *Borrelia* species whose geographic distributions differ. Thus, infection with *Borrelia burgdorferi sensu stricto,* which rarely features skin lesions, is the only cause of Lyme disease in North America, whereas in Europe infections with *Borrelia afzelii* (often affecting the skin) and *Borrelia garinii* dominate.[15] However, serologic evidence of previous *Borrelia*

Table 2. Crude odds ratios (ORs) and 95% confidence intervals (CIs) for the association between *Borrelia* infection and risk of non-Hodgkin lymphoma (NHL) overall and NHL subtype

	All NHLs		DLBCL		CLL		FL		MCL		MZL		LPL		TCL	
	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)	No.	OR* (95% CI)
Tick bite,† self-reported																
Ever‡	1248	1.0 (0.9-1.1)	319	0.99 (0.8-1.2)	318	1.1 (0.9-1.3)	224	0.9 (0.8-1.1)	65	1.0 (0.7-1.5)	46	1.01 (0.7-1.5)	51	1.2 (0.8-1.8)	71	0.8 (0.6-1.1)
$P_{homogeneity}$		0.90		0.92		0.49		0.35		0.83		0.95		0.42		0.17
***Borrelia* infection,† self-reported**																
Ever§	102	1.3 (0.96-1.8)	26	1.3 (0.8-2.1)	23	1.2 (0.8-2.0)	19	1.3 (0.8-2.2)	9	2.5 (1.2-5.1)	0	–	5	1.8 (0.7-4.7)	8	1.8 (0.8-3.8)
$P_{homogeneity}$		0.09		0.24		0.40		0.35		0.03		–		0.26		0.16
Anti-*Borrelia* antibodies‖																
Positive¶	73	1.3 (0.9-2.0)	18	1.4 (0.8-2.5)	23	1.0 (0.6-1.8)	13	1.1 (0.6-2.1)	12	3.6 (1.8-7.4)	2	1.1 (0.3-4.6)	0	–	3	0.7 (0.2-2.5)
$P_{homogeneity}$		0.15		0.24		0.87		0.79		0.001		0.94		–		0.61

NHL indicates non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; LPL, lymphoplasmacytic lymphoma; and TCL, T-cell lymphoma.

*Adjusted for sex, age in 10-year intervals, and country of residence (matching variables).

†Including answers of all SCALE participants (3055 NHL cases and 3187 matched controls).

‡Reference: never have had a tick bite.

§Reference: never have had an injection with *B burgdorferi*.

‖*Borrelia*-specific IgG antibodies were analyzed in a case-control study subset including serum samples of 1579 cases with NHL and 1358 matched controls; in 6 participants (2 cases and 4 controls), serologic analyses yielded inconclusive results and were excluded.

¶Reference: negative for *Borrelia*-specific IgG antibodies in serum.

infection was found in chart reviews in 10 of 23 patients with PCBCL in a recent American investigation.[28]

In our investigation, there was little solid evidence of a predilection for the skin among the lymphomas that could be suspected to be causally linked with *Borrelia* infection. Rather, we found evidence to suggest an increased risk of MCLs among those with a *Borrelia* infection history. We recently demonstrated the presence of *Borrelia* DNA in 2 nodal lymphomas, 1 of which was an MCL, diagnosed in patients with a documented history of *Borrelia* infection.[16] However, previous studies, albeit with a focus on PCBCL, have suggested associations with risk of MZL, FL, DLBCL, and T-cell lymphomas.[12,24,28] Moreover, *Borrelia* DNA has also been detected in cutaneous B-CLL infiltrates,[29] analogous to one of our seropositive patients.

The suspected mechanism for *Borrelia*-induced lymphoma development is chronic antigen-dependent immunostimulation triggering sustained lymphoid proliferation with oligoclonal and, ultimately, monoclonal B-cell selection, analogous to *Helicobacter pylori*–associated gastric MALT lymphoma.[19,30] This mechanism would favor lymphoma subtypes such as MALT lymphoma originating from germinal center or post–germinal center B cells.[19] The specificity of the *Borrelia* association with MCL in our study is therefore somewhat surprising, considering its predominantly pregerminal origin. Still, we have previously described *Borrelia* DNA in a case of nodal MCL, and others have reported hepatitis C virus RNA in MCL that, moreover, responded with complete regression after antiviral treatment.[31] In addition, Jares et al mentioned in a recent review that 15% to 40% of MCLs may carry somatic hypermutations suggesting that some tumors originate in cells under the influence of the germinal center environment.[32] Accordingly, the increased MCL risk seems biologically plausible but warrants confirmation.

Interestingly, the association between MCL risk and *Borrelia* seropositivity was also seen in patients with no recollection of *Borrelia* infection. Asymptomatic individuals with positive *Borrelia* serology have been observed previously,[33] and the immune response may differ between patients with or without clinical Lyme borreliosis.[34] Still, no differences in the Th1-dominated immune response, supposedly predominant in the eradication of *Borrelia* spirochetes, were observed between *Borrelia*-positive asymptomatic individuals and patients with clinical *Borrelia* infection in a Swedish study.[34]

Although not fully understood, the *Borrelia* spirochete has different strategies to escape the host immune system and maintain the infection. These may include antigenic variation of its surface membrane, binding to complement control proteins, and intracellular persistence (reviewed in Singh and Girschick[35]). In one study, patients with acute Lyme borreliosis manifesting as erythema migrans (EM) initially showed high levels of the Th1 cytokine interferon-gamma (IFN-gamma), followed by increased levels of the Th2 cytokine interleukin-4 (IL-4) after *Borrelia* clearance. In contrast, patients with chronic *Borrelia* infection manifesting as ACA had persistently high IFN-gamma levels but no increase in IL-4.[36] Another study corroborated the cytokine findings in EM, but observed high IL-4 levels and very little or no IFN-gamma expression in ACA patients.[37] Both studies, however, concluded that the expression of IFN-gamma seemed particularly important for the control and resolution of a *Borrelia* infection. This finding is relevant to the *Borrelia*-lymphoma association because a Th1-dominated immune response has been linked to increased risk of other chronic inflammatory diseases,[38] which in turn have been linked to elevated NHL risk.[39,40] Furthermore, a Th1-dominated

immune response has been observed in *Helicobacter pylori*–positive gastric MALT lymphomas.[41]

The strengths of our study include its uniquely large size, the population-based design, the rapid and complete case ascertainment of incident cases of NHL, the uniform classification of NHL subtypes according to the *World Health Organization Classification of Tumors,*[19] and blood samples from a high percentage of NHL cases and matched controls. The positive association between self-reported *Borrelia* infection and MCL could theoretically have resulted from recall bias (ie, that cases tend to better recall specific exposures than controls). However, the hypothesis of an association between *Borrelia* infection and risk of NHL or one of its subtypes is not well known, which makes recall bias less likely. Moreover, the validity of the association based on self-reported *Borrelia* infection history was supported by the serologic analyses. Hence, the association with *Borrelia* seropositivity pertained to both those with and those without self-reported history of *Borrelia* infection.

The relatively low seroprevalence of anti-*Borrelia* antibodies observed in our investigation accords with the regional variation of *Borrelia* genospecies and differences in the manifestation of Lyme borreliosis.[42,43] Incidence rates of Lyme borreliosis vary geographically; however, because it is not a notifiable disease in Europe, reliable epidemiologic data are limited. The seroprevalence of anti-*Borrelia* antibodies in healthy blood donors has been estimated to be 2% in Denmark[44] and 19% in southern Sweden.[45]

The serologic testing for Lyme borreliosis has some limitations, including a low diagnostic sensitivity in early disease due to slow antibody response, and a degree of IgG cross-reactivity, especially with *Treponema pallidum*.[44] Moreover, in long-term follow-up studies, a decrease in IgG levels has been observed in patients treated for either chronic cutaneous borreliosis or EM.[46,47] However, the flagella ELISA used in our study has a specificity of 95%, as estimated from a panel of sera from 200 Danish blood donors with no experience of tick bites or signs and symptoms of *Borrelia* infection, and a sensitivity between 79% and 100% correlating with the stage of *Borrelia* infection.[48-50] Finally, any test-related bias would presumably affect cases and controls similarly, which would attenuate any true association. When evaluating possible biases related to serum availability according to tick bite and self-reported *Borrelia* infection, self-reported *Borrelia* infection was not associated with blood sampling in cases or controls, whereas controls but not cases with history of tick bites had given blood more often than those without (data not shown). This bias is also unlikely to explain our observations. We cannot completely rule out chance findings due to the multiple comparisons. However, we note that the observation of an association between *Borrelia* infection and MCL was consistent in 2 independent analyses of persons, one with self-reported and one with serologic evidence of *Borrelia* infection, which is unlikely to be explained by chance alone.

In conclusion, for the first time, we found evidence to suggest an association between *Borrelia* infection and risk of mantle cell lymphoma. This novel observation requires confirmation, for example, from studies testing for the presence of *Borrelia* DNA in tumor tissue or from investigations nested in cohorts with access to serologic, register, and/or interview information about *Borrelia* infection.

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Authorship

Contribution: The study was designed by M.M., H.-O.A., B.G., L.M., M.H., H.H., K.E.S., E.T.C., and C.S., who secured funding and also collected data with assistance from G.R.; statistical analyses were performed and interpreted by C.S., K.R., H.H., and M.M.; C.S. wrote the first paper, which was critically revised and approved by all authors.

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