Haematologica 2000; 85:147-153
original paper

Malignant Lymphomas

Prevalence of *Helicobacter pylori* and hepatitis C virus infections among non-Hodgkin's lymphoma patients in Southern Switzerland

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ABSTRACT

Background and Objectives. **Several recent studies have reported a high rate of previous hepatitis C virus (HCV) infection in patients with non-Hodgkin's lymphoma (NHL). However, it appears that there are marked geographical differences in the prevalence of HCV among NHL patients. There is further controversy concerning a possible pathogenetic link between HCV and certain histologic lymphoma subtypes, in particular MALT lymphomas, and it has recently been speculated that HCV might be involved in the multistep process of gastric lymphoma genesis, in addition to the well established role of chronic *Helicobacter pylori* infection. The aim of this study was to investigate the prevalence of HCV and *H. pylori* infections in patients with B-cell NHL in Southern Switzerland.**

Design and Methods. **One hundred and eighty newly diagnosed HIV-negative B-cell NHL patients, consecutively seen at a referral oncology center in Southern Switzerland between 1990 and 1995 were prospectively studied. A microparticle enzyme immunoassay was used to detect antibodies to HCV. Serologic determination of HCV genotype was done by the Murex method. The quantitative detection of IgG anti-*H. pylori* was performed by the Biorad GAP test.**

Results. **Infection with HCV was detected in 17/180 patients (9.4%; 95% C.I., 6%-15%). This prevalence is significantly higher than that observed in a large survey of 5424 new blood donors from the same area tested in 1992-97 (0.9%; 95% C.I., 0.7-1.2). Neither histologic subtypes nor specific extranodal presentations of NHL were associated with a higher prevalence of HCV. HCV serotype 2 (corresponding to genotypes 2a-c) was the most common. HCV infection was significantly associated with a shorter progression-free survival at both univariate and multivariate analysis. Anti-*Helicobacter* antibodies were detected in 81/180 patients (45%; 95% C.I., 38%-53%) and *H. pylori* infection was significantly associated with the development of primary lymphomas of the stomach.**

Interpretation and Conclusions. **A high prevalence of HCV infection was detected in NHL lymphoma patients and was associated with a shorter time to lymphoma progression. HCV infection was not correlated with primary gastric presentation or with MALT-type histology. Our findings further support the key role of *H.pylori* infection in the pathogenesis of primary gastric lymphoma of MALT-type. The possible role of HCV in the pathogenesis of NHL should be further investigated.**

Key words: HCV, *Helicobacter pylori*, MALT lymphoma, extranodal marginal zone B-cell lymphoma, non-Hodgkin's lymphoma, hepatitis C

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Lymphomagenesis is a multifactorial process in which genetic, environmental, and infectious factors can be involved. Several specific viruses such as Epstein-Barr virus (EBV), human herpes viruses type 6 (HHV-6) and type 8 (HHV-8), and human T-cell lymphoma viruses type 1 (HTLV-1) and type 2 (HTLV-2) have been proposed as causative agents of particular histologic subtypes of non-Hodgkin's lymphoma (NHL).[1]

Hepatitis C virus (HCV), which is both hepato- and lymphotropic, has been associated with a wide constellation of extrahepatic immune-mediated conditions, including cutaneous, renal, endocrine and hematologic disorders.[2] Special attention has recently been devoted to its link with monoclonal gammopathies, and particularly with mixed cryoglobulinemia type II, which is currently considered to be an indolent clonal B-cell lymphoproliferative disorder which sometimes may evolve into a frank malignant B-cell NHL.[3,4] Considerable evidence has been accumulating in favor of HCV being directly involved in the pathogenesis of mixed cryoglobulinemia type II.[5–7] This finding prompted the performance of several studies searching for a direct relationship between HCV and NHL, which produced conflicting results. A strong association between HCV infection and NHL has been suggested by epidemiologic studies from Italy, Asia and the USA,[8-20] but other studies in other European countries and the USA have failed to confirm this association; apparently there are marked

geographical differences in the prevalence of HCV among NHL patients.[21-26] There is further controversy about the possibility of a pathogenetic link between HCV and certain histologic lymphoma subtypes, in particular the lymphoplasmacytoid lymphoma/immunocytoma and the marginal zone B-cell lymphomas.[14,16,18,27] Since most low-grade gastric lymphomas are of marginal zone B-cell type (MALT lymphomas), it has recently been speculated that HCV may also be relevant in the multistep process of gastric lymphomagenesis and that it might be implicated in the additional neoplasms seen in some gastric lymphoma series.[28] This hypothesis has not yet been confirmed and gastric lymphomas are still considered to be strongly associated with chronic *Helicobacter pylori* infection.[29]

On the basis of these observations, we sought to determine the prevalence of infection with HCV in patients affected by B-cell NHL in our geographical area. Our analysis was extended to all newly-diagnosed NHL patients seen at our institution between 1990 and 1995 for whom we had both complete follow-up information and a frozen serum sample taken at the time of their diagnoses of lymphoma. After the suggestion of a possible pathogenetic role for HCV in gastric lymphomas, we extended our study to a search for anti-*Helicobacter* antibodies in this series of patients.

Design and Methods

Patients

Initial serum samples (collected at the time of NHL diagnosis and stored at –20°C) were available from 180 patients with non-Hodgkin's lymphomas, consecutively diagnosed and treated in our institution between January 1990 and December 1995. None of the patients had a history of prior active liver disease or prior mixed cryoglobulinemia. All cases have been prospectively registered, with inclusion of data regarding clinical staging, biological pre-treatment values, therapy and survival. Routine staging procedures included chest X-rays and/or computed tomography (CT) of the chest; abdominal and pelvic ultrasound and/or CT; bone marrow aspirate and biopsy; full blood counts, tests of renal and liver functions, and measurement of serum lactate dehydrogenase (LDH) level. Clinical stage was defined according to the Ann Arbor staging system. Primary gastric non-Hodgkin's lymphomas were additionally staged according to the system for gastrointestinal NHL proposed by Rohatiner *et al.*[30] Performance status (PS) was evaluated by the *Eastern Cooperative Oncology Group (ECOG)* scale. Bulky disease was defined as a mass of >10 cm in diameter. All cases were histologically reviewed and re-classified according to the REAL classification of lymphoid neoplasms.[31]

Serum testing

Serum samples from each patient were screened for both HCV antibodies and IgG antibodies to *Helicobacter pylori*. All samples were retrieved and placed into tubes labeled individually with identification numbers, and blinded analyses were then performed. A microparticle enzyme immunoassay (MEIA) was used for qualitative detection of anti-HCV IgG (AxSYM, Abbott diagnostic division, Germany). All seropositive results were confirmed by immunoblot assay (Wellcozyme, Murex Diagnostic, UK). For the quantitative (GAP test, Biorad, USA) detection of anti-*Helicobacter pylori* IgG, each serum sample was run in duplicate and equivocal results were retested using another ELISA kit (Premier Hp. EIA, Meridian). All analyses were performed in accord once with the instructions provided by the suppliers.

Serologic determination of HCV genotype was performed by measuring type-specific antibodies to NS4-derived peptide antigens by the Murex HCV serotyping 1-6 assay. Results obtained by this indirect-typing method correlate well with those of the more complex and expensive genotyping assay by RT-PCR. Moreover, the method allows detection of antibodies to past infection and does not depend on present HCV viremia.[32]

Statistical methods

Overall survival (OS) was defined as the time from the day of diagnosis until death of any cause or until the last follow-up. Time to progression (TTP) was measured as the time from the diagnosis until progression, relapse after response or death from lymphoma or its therapy. Actuarial survival probabilities were calculated using the life-table method. Survival curves were estimated using the Kaplan-Meier method and differences between curves were analyzed using the log-rank test. Binomial exact 95% confidence intervals (95% CI) were calculated for percentages. The chi-square test was used for testing associations in two-way tables whenever appropriate. The Cox proportional hazard model was used for multivariate analysis and estimation of relative risk (hazard ratio) and its confidence interval. p values of 0.05 or less were considered to indicate statistical significance. The STATA 3.0 software package (Computing Resource Centre, Santa Monica, CA, USA) was used for all statistical procedures.

Results

Detailed clinical characteristics of the 180 analyzed patients are shown in Table 1. Anti-HCV antibodies were detected in 17/180 (9.4%, 95% CI 6-15%) patients. This HCV prevalence was significantly higher (p<0.001) than the 0.9% (95% CI 0.7-1.2%) found in a survey of 5,424 new blood donors from the same area tested between 1992 and 1997 (Swiss Red Cross Transfusional Medicine Service for Canton Ticino, registry data). The median age was 61 years in the series as a whole, with a range of 16-87 years. Clinical symptoms of mixed cryoglobulinemia were not reported in the HCV-positive patients and the presence of serum cryoglobulins was not tested at diagnosis. At univariate analysis HCV infection was significantly associated with elevated β_2-microglobulin levels (p=0.02), and a significant association was also found with the presence of B-symptoms (p=0.04).

None of the other variables commonly predicting the outcome of lymphoma patients was significantly associated with detection of circulating anti-HCV antibodies (Table 1). Moreover, no histologic subtype was significantly associated with a higher preva-

Table 1. Characteristics of NHL patients (n=180) according to their HCV status.

Features	*HCV–* *n*	*(%)*	*HCV+* *n*	*(%)*	*p*
Age					
60 years (n=88)	79	(90%)	84	(91%)	
>60 years (n=92)	9	(10%)	8	(9%)	n.s.
Sex					
females (n=79)	71	(90%)	8	(10%)	
males (n=101)	92	(91%)	9	(9%)	n.s.
Performance status					
ECOG score 1 (n=160)	147	(92%)	13	(8%)	
ECOG score >1 (n=20)	16	(80%)	4	(20%)	n.s.
Stage					
I-II (n=65)	61	(94%)	4	(6%)	
III-IV (n=115)	102	(89%)	13	(11%)	n.s.
B-symptoms					
absent (n=141)	131	(93%)	10	(7%)	
present (n=39)	32	(82%)	7	(18%)	0.04
Bulky disease					
absent (n=157)	141	(90%)	16	(10%)	
present (n=23)	22	(96%)	8	(4%)	n.s.
Serum LDH					
normal (n=113)	104	(92%)	9	(8%)	
elevated (n=67)	59	(88%)	8	(12%)	n.s.
Serum β_2 microglobulin*					
normal (n=91)	87	(96%)	4	(4%)	
elevated (n=66)	56	(85%)	10	(15%)	0.02
Number of extranodal sites					
1 (n=136)	123	(90%)	13	(10%)	
>1 (n=44)	40	(91%)	4	(9%)	n.s.
International Prognostic Index					
low to low/intermediate risk (n=121)	111	(92%)	10	(8%)	
intermediate/high to high risk (n=59)	52	(88%)	7	(12%)	n.s.
Bone marrow involvement					
absent (n=93)	87	(94%)	6	(6%)	
present (n=87)	76	(87%)	11	(13%)	n.s.
Hepatomegaly					
absent (n=169)	154	(91%)	15	(9%)	
present (n=11)	9	(82%)	2	(18%)	n.s.
Spleen enlargement					
absent (n=133)	120	(90%)	13	(10%)	
present (n=47)	43	(91%)	4	(9%)	n.s.
Gastric involvement					
absent (n=157)	142	(90%)	15	(10%)	
present (n=23)	21	(91%)	2	(9%)	n.s.
Helicobacter pylori infection					
absent (n=99)	91	(92%)	8	(8%)	
present (n=81)	72	(89%)	9	(11%)	n.s.

** β_2-microglobulin serum levels were determined at diagnosis in 157/180 patients only.*

lence of HCV infection in our series (Table 2). HCV serotype 2 (corresponding to genotypes 2a-c) was the most common (Table 3).

The presence of HCV was significantly (p= 0.0066) associated with a reduced time to progression (TTP: 47% vs. 16% at 3 years, median 27 vs. 8 months) (Figure 1) but not with the overall survival (3 years, OS: 71% vs. 68%, median not reached) duration (Figure 2). Despite the limits of a multivariate analysis related to the small number of HCV-infected cases, the prognostic impact on TTP was confirmed in a Cox regression model (Table 4) after correction for the β_2-microglobulin level, for the presence of B-symptoms and for the international prognostic index (which is determined for the individual patients on the basis of age, stage, performance status, serum LDH level, and number of extranodal localizations).[33] The model as a whole was statistically significant.

Anti-*Helicobacter pylori* antibodies were detected in 81/180 (45%, 95% CI 38-53%) patients. Eighteen of 180 patients (10%, 95% CI 6.5-15%) had a primary gastric lymphoma. Of the 18 patients with gastric lymphoma 12 had marginal zone B-cell lymphomas (low-grade MALT lymphomas), 6 diffuse large-cell lymphomas (5 of 6 of whom showed residual foci of low-grade MALT lymphoma suggesting a derivation from a transformed marginal zone B-cell lymphoma).

Table 2. Prevalence of anti-*H. pylori* and HCV antibodies in the different subtypes of non-Hodgkin's lymphomas classified according to the R.E.A.L. classification.

	n	*anti-H.pylori Ab (%)*	*95% CI*	*p*	*Anti-HCV Ab (%)*	*95% CI*	*p*
A	31	15 (48)	30-67		3 (10)	2-26	
B	13	9 (69)	39-91		1 (8)	0-36	
C	46	19 (41)	27-57		3 (6.5)	1-18	
D	24	10 (42)	22-63		2 (8)	1-27	
E*	58	27 (47)	33-60		8 (14)	6-25	
F	8	1 (13)	0-53		0	–	
Total	180	81(45)	38-53	n.s.	17 (9)	6-15	n.s.

*CI: confidence interval; Ab: antibodies; p value = chi square. A: small B-lymphocytic lymphoma and lymphoplasmocytoid/immunocytoma; B: marginal zone B-cell lymphoma (low-grade MALT type); C: follicle center lymphoma; D: mantle cell lymphoma; E: diffuse large B-cell lymphoma; F: other high-grade lymphomas (Burkitt's/lymphoblastic). *Including high-grade MALT lymphoma.*

Table 3. Prevalence of HCV serotypes among HCV-infected patients with NHL in Southern Switzerland.§

HCV type (Murex serotype assay)	*n (%)*	*[95% C.I.]*
1	2 (13.3%)	[2%-40%]
1+2	1 (6.6%)	[0.2%-32%]
2	6 (40%)	[16-68]
3	0	–
4	1 (6.6%)	[0.2%-32%]
5	1 (6.6%)	[0.2%-32%]
6	0	–
NR*	2 (13.3%)	[2%-40%]
NT°	2 (13.3%)	[2%-40%]

*§Only 15 of 17 seropositive patients analyzed due to the lack of remaining serum in 2 cases. *Non-reactive; °no type specific antibodies.*

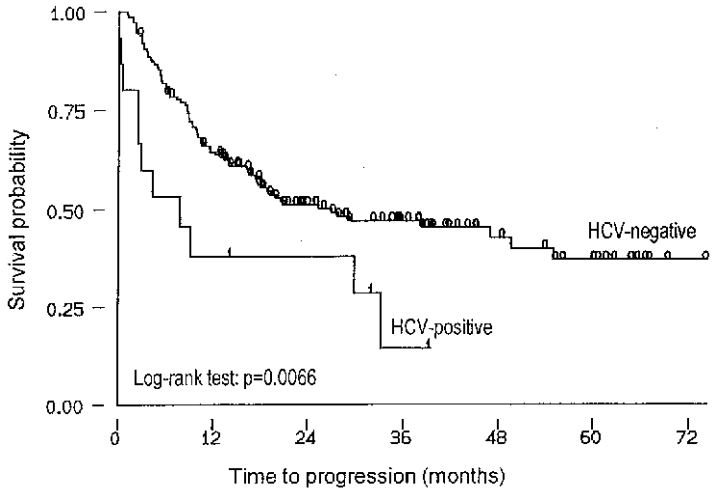

Figure 1. Time to progression by HCV serology in 149 NHL patients, who achieved a complete or partial response. TTP was significantly better for HCV-negative patients (47% vs. 16% at 3 years; median 27 vs. 8 months).

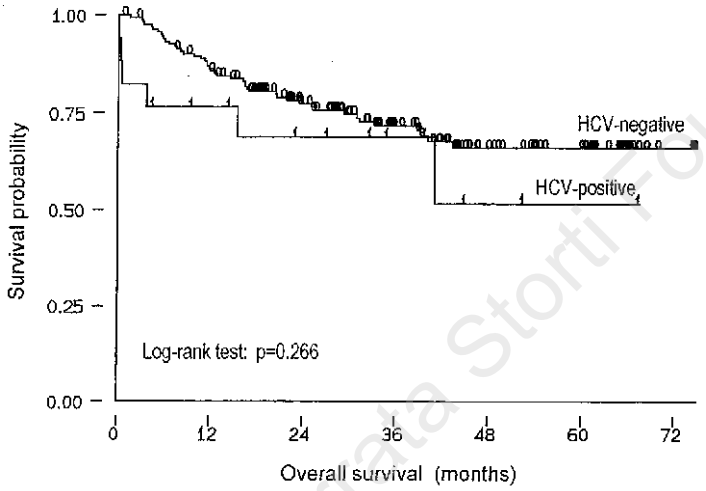

Figure 2. Overall survival by HCV serology in a series of 180 patients with NHL from Southern Switzerland. Survival was not significantly different in the two groups (3-year OS, 71% vs. 68%, median not reached).

Anti-*Helicobacter pylori* antibodies were detected in 13/18 (72%, 95% CI 47-90%) gastric lymphoma patients (7/12 low-grade MALT lymphomas and 4/6 DLCLs) and in 68/162 (42%, 95% CI 34-50%) non-gastric lymphoma patients (Table 5). This association of *Helicobacter pylori* infection with primary lymphomas of the stomach was statistically significant (p=0.014). Anti-HCV antibodies were present in the serum of 2/18 (11.1%, 95% CI 1-35%) primary gastric lymphoma patients and in the serum of 15/162 (9.2%, C.I. 5-15%) non-gastric lymphoma patients; HCV infection was not correlated with either MALT-type histology or the gastric lymphoma localization.

Fourteen of 180 patients had additional neoplasms (8%, 95% CI 4-13%). Of these, 6 were diagnosed at least 1 year prior to, 2 concomitantly with (i.e., within 1 year), and 6 subsequent to the lymphoma diagnoses. Eleven had solid tumors: 3 lung, 3 breast, 1 head and neck, 1 melanoma, 1 liver cancer, 1 rectal adenocarcinoma and 1 endometrial adenocarcinoma. Three cases had hematologic neoplasms: 1 hairy cell leukemia and 2 myelodysplastic syndromes. The presence of additional tumors was significantly associated with the primary gastric localization of lymphoma (p=0.016), but not with the presence in the serum of either anti-HCV or anti-*Helicobacter pylori* antibodies.

Discussion

Despite the lack of a direct demonstration of the role of HCV in lymphomagenesis, several lines of evidence appear to support this hypothesis. The epidemiological association between HCV infection and non-Hodgkin's lymphomas has been explored in populations of various countries (Table 6), but no data from Switzerland on this subject have been published before now.

Analogous to the results of several epidemiological studies from Italy, Japan and the U.S., the prevalence of HCV infection in NHL patients appears much higher than in the general population of our geographical area, at least on the basis of the screening of voluntary blood donors, since no other data on the general population are available. It could be argued that

Table 4. Multivariate analysis (Cox proportional hazard model) of time to progression (TTP).

Variable	*Hazard ratio*	*Standard error*	*95% C.I.*	*p value*
HCV	2.17	0.80	1.04 - 4.52	0.039
IPI	1.59	0.22	1.21 - 2.10	0.001
B-symptoms	1.23	0.35	0.70 - 2.15	0.471
β_2-microglobulin	1.30	0.38	0.72 - 2.33	0.380

The relative risk of progression (hazard ratio) is significantly higher for HCV-positive patients controlling for the international prognostic index (IPI), the presence of systemic symptoms and the β_2-microglobulin serum level. Patients with poorer IPI are more likely to have a shorter TTP; B-symptoms and β_2-microglobulin do not show any significant impact on the progression risk. The model as a whole was statistically significant.

Table 5. Prevalence of HCV and *H. pylori* infections in patients with primary lymphoma of the stomach.

	Gastric lymphoma (n=18)	*Non-gastric lymphoma (n=162)*	*p value (chi-square)*
	No. of seropositive patients		
Anti- HCV	2 (11%, 95%C.I., 1%-35%)	15 (9%, 95%C.I., 6%-15%)	n.s.
Anti- H.pylori	13 (72%, 95%C.I., 47%-90%)	68 (42%, 95%C.I., 34%-50%)	0.014

blood donors may not represent a proper control group since it is known that the prevalence of HCV in the whole population can be underestimated by checking only blood donors. It is, therefore, worth mentioning that even in a general population screening conducted in Northern Italy (in an almost neighboring area, with a population in which ethnic and environmental factors are likely to be very close to those in Southern Switzerland) the prevalence of HCV was clearly lower than the one we found in our lymphoma patients.[34]

This observation supports the hypothesis that HCV infection plays a role in lymphomagenesis, although our data are not adequate to define the type of mechanism behind the increased risk of lymphoma in HCV-infected patients. The above mentioned possible genetic and geographical closeness between Southern Switzerland and Northern Italy might explain the similarity of our results to those reported by several Italian groups, whilst negative surveys have been reported in other European countries (Table 6).

In our series no histologic subtype was significantly associated with a higher prevalence of HCV infection. In particular, the detection of circulating anti-HCV antibodies was not correlated with either MALT-type histology or the gastric lymphoma localization. These results contradict the conclusions of previous reports suggesting a role for HCV infection in the multistep pathogenesis of low-grade lymphomas of MALT type.[28,35] Conversely, the statistically significant association between the presence of anti-*H. pylori* antibodies and primary lymphomas of the stomach further supports a key role for *H. pylori* infection in the pathogenesis of primary gastric lymphoma of MALT type.[29]

Table 6. Prevalence of HCV antibodies in the most relevant reported series.

Reference	*Country*	*No. of patients*	*HCV infection rate (%*
Pozzato *et al*.[15]	Italy	198	28
Silvestri *et al*.[16]	Italy	311	9
Luppi *et al*.[12]	Italy	157	22
Pioltelli *et al*.[14]	Italy	126	21
DeVita *et al*.[20]	Italy	157	22
Mazzaro *et al*.[13]	Italy	199	28
King *et al*.[23]	USA	73	1.4
Kashyap *et al*.[26]	USA	312	7
Zuckerman *et al*.[18]	USA	120	22
Yoshikawa *et al*.[17]	Japan	55	16
Izumi *et al*.[11]	Japan	25	16
Hanley *et al*.[22]	UK	38	0
Brind *et al*.[21]	UK	63	0
McColl *et al*.[24]	UK	72	0
Ellenrieder *et al*.[25]	Germany	69	4.3
Germanidis *et al*.[44]	France	201	2
Vallisa *et al*.[45]	Italy	175	37
Present series	Switzerland	180	9.4

An increased number of additional neoplasms was found in the group of patients affected by primary gastric lymphomas; however, we found no statistically significant association between additional neoplasms and HCV or *H. pylori* infection. This association of additional neoplasms with the gastric localization of lymphoma remains unexplained. It might be related to the genetic instability described in MALT lymphomas,[36] but this latter finding is still controversial[37] and, in general, microsatellite instability appears to be very rare in NHL.[38]

Univariate and multivariate analyses of the survival data showed the presence of HCV to be associated with a significantly shorter TTP (Table 4, Figure 1). This finding, never before reported, may be attributable to the HCV-related morbidity and perhaps to difficulties in administering full doses of intensive chemotherapy, which can be highly toxic in the presence of chronic liver disease. However, there was no significant difference in overall survival duration among our patients in relation to the presence of HCV infection. A longer follow-up will probably establish whether the differences observed in TTP reflect differences in OS.

Serologic determination of HCV genotype by measurement of genotype-specific antibodies directed to NS4-derived peptide antigens showed a greater prevalence of serotype 2, corresponding to genotypes 2a-c of the nomenclature proposed by Simmonds *et al*.[39] This result, analogous to those in previous reports,[40,41] further suggests that group 2 genotypes might be involved in the pathogenesis of lymphoproliferative

and autoimmune disorders. An altered immune reactivity is indeed present in HCV patients, in whom elevated serum levels of soluble immune factors can often be found,[42,43] and the elevated β_2-microglobulin levels we found in patients carrying anti-HCV antibodies are probably an expression of this HCV-related disturbance of the inflammatory-immune system.

The possible role of HCV in the pathogenesis of B-cell NHL warrants further investigations.

Contributions and Acknowledgments

EZ and ER were the main investigators, designed the study, managed the data, and with AC, FB , and IR reviewed the literature and wrote the manuscript. NM contributed to the study design and to the laboratory analyses. DC produced the data on the control group of blood donors. EP was the pathologist who reviewed all the cases. FC and JCP took part in the conception of the study and interpretation of results, and gave final approval of the manuscript to be submitted.

The criteria for the order of authors' names is based on their contribution as delineated above.

Funding

This work was partially supported by Swiss National Science Foundation grants 32-45993.95 (E.Z.), and 31-45914.95 (J.C.P.) and by the Krebsforschung Schweiz and the Schweizerische Krebsliga (grants KFS 233-12-1995 and SKL 266-1-1996).

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received July 7, 1999; accepted November 4, 1999.

Potential Implications for clinical practice

- Analogous to gastric MALT lymphomas – in which the successful introduction of antibiotic therapy was based on the evidence of a pathogenetic link between Helicobacter pylori infection and tumor development – better comprehension of the role played by other infectious agents in the pathogenesis of human tumors and, in particular, of non-Hodgkin's lymphomas might lead to new therapeutic strategies being defined.

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