

# Infection with Hepatitis B and C Viruses and Risk of Lymphoid Malignancies in the European Prospective Investigation into Cancer and Nutrition (EPIC)

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## Abstract

**Background:** Case-control studies suggested a moderate, but consistent, association of hepatitis C virus (HCV) infection with lymphoid tissue malignancies, especially non-Hodgkin lymphoma (NHL). More limited data suggested that hepatitis B virus (HBV) infection might also be associated with NHL. However, prospective studies on the topic are few.

**Methods:** A nested case-control study was conducted in eight countries participating in the EPIC prospective study. Seven hundred thirty-nine incident cases of NHL, 238 multiple myeloma (MM), and 46 Hodgkin lymphoma (HL) were matched with 2,028 controls. Seropositivity to anti-HCV, anti-HBc, and HBsAg was evaluated and conditional logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) for NHL, MM, or HL, and their combination.

**Results:** Anti-HCV seropositivity among controls in different countries ranged from 0% to 5.3%; HBsAg from 0% to 2.7%; and anti-HBc from 1.9% to 45.9%. Similar nonsignificant associations were found with seropositivity to HBsAg for NHL (OR = 1.78; 95% CI: 0.78–4.04), MM (OR = 4.00; 95% CI: 1.00–16.0), and HL (OR = 2.00; 95% CI: 0.13–32.0). The association between HBsAg and the combination of NHL, MM, and HL (OR = 2.21; 95% CI: 1.12–4.33) was similar for cancer diagnosed less than 3 and 3 or more years after blood collection. No significant association was found between anti-HCV and NHL, MM, or HL risk, but the corresponding CIs were very broad.

**Conclusions:** Chronic HBV infection may increase the risk of lymphoid malignancies among healthy European volunteers.

**Impact:** Treatment directed at control of HBV infection should be evaluated in HBsAg-seropositive patients with lymphoid tissue malignancies. *Cancer Epidemiol Biomarkers Prev*; 20(1); 208–14. ©2011 AACR.

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## Introduction

Approximately 74,000, 32,000, and 12,000 new cases of non-Hodgkin lymphoma (NHL), multiple myeloma (MM), and Hodgkin lymphoma (HL), respectively, were estimated to have occurred in the European Union in 2008 (1). When combined, these 3 lymphoid tissue malignancies, which arise in the vast majority from B-cell lymphocytes (2), represent the fourth most frequent cancer in the European Union. Upward incidence trends for NHL (3) and MM (4) were observed in most European countries and the United States until the mid-1990s when incidence rates leveled off. The pattern of incidence trends for HL was less clear, with a general tendency to remain stable or to decline in the last decades in many European countries (5) and the United States (6).

Immunodeficiency and infection with certain viruses [Epstein-Barr, T-cell leukemia/lymphoma, Kaposi sarcoma herpes virus, and human immunodeficiency virus (HIV)] and bacteria (*Helicobacter pylori*) are the best documented causes of lymphoid tissue malignancies, although they account for a relatively small fraction of these diseases and are typically associated with specific histological subtypes or localizations (7).

A moderate, but consistent, association of hepatitis C virus (HCV) infection with NHL risk has gradually emerged over the last 2 decades (8–10) and led the World Health Organization (WHO)/International Agency for Research on Cancer (IARC) Monograph Working Group to consider that HCV does cause NHL, especially B-cell lymphoma (11). Data on the association of HCV with MM and HL risk are scantier, but findings were similar to those for NHL (9). There is also evidence of an association between hepatitis B virus (HBV) infection and NHL risk (12), although there are fewer studies on this topic (11) than on the association with HCV. Notably, an approximately 2-fold increased NHL risk has recently been reported among chronic HBV carriers in a large cohort study from South Korea (13).

With the exception of the South Korean study, most evidence on the relationship between HCV or HBV and NHL, MM, and HL derives from case-control studies (9, 12). Therefore, we took advantage of the European Prospective Investigation into Cancer and Nutrition (EPIC) to further elucidate the issue.

## Methods

EPIC is a large prospective cohort study with more than 500,000 participants enrolled from 23 centers in Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom (14). Eligibility in the EPIC study was based essentially on geographic and administrative boundaries, although some special population groups were also included. The Utrecht subcohort in the Netherlands, for instance, was based on women who underwent breast cancer screening, and the French subcohort was based on women who were

members of the health insurance system or state-school employees. In addition, all 5 Spanish subcohorts, and the Turin and Ragusa subcohorts in Italy, included high proportions (46%–100%) of blood donors. Participation rates are not available from all participating centers (14). Between 1991 and 2000, standardized lifestyle and personal history questionnaires were collected (median year at enrolment: 1995; 5th–95th percentile: 1993–1998). Blood samples were also collected at enrolment and fractionated into aliquots of serum, plasma, red blood cells, and buffy coats. One half of each aliquot was stored locally at the corresponding EPIC center, and the other half at the IARC (Lyon, France, storage at  $-196^{\circ}\text{C}$  in liquid nitrogen), with the exception of Denmark (storage at  $-150^{\circ}\text{C}$  in nitrogen vapor) and Sweden (storage in  $-80^{\circ}\text{C}$  freezer). Ethical approval for this study has been obtained from all participating centers and the IARC Internal Review Board.

## Follow-up for cancer incidence and vital status

Vital status follow-up in EPIC (99% complete) is collected through record linkage with regional and/or national mortality registries in all countries except Germany and Greece, where data are collected actively. Cancer incidence is determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom; complete, for the present report, up to December 2004) or via a combination of methods, including the use of health insurance records, contacts with pathology registries, and active follow-up through participants and their next of kin (France, Germany, and Greece; complete up to December 2006).

The present study does not include data from France, due to incomplete coding for lymphoid tissue malignancies, or from Norway (only 9 NHLs and no MM and HL with blood samples available). In addition, 25% and 75% participants from Italy and Spain, respectively, were excluded because they were blood donors and therefore prescreened for exposure, or seropositivity, to markers of HCV and HBV infection.

## Nested case-control design and participant selection

Lymphoid tissue malignancies reported in the EPIC study were initially classified according to the second revision of the International Classification of Diseases for Oncology (ICD-O-2). They were subsequently recoded according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Third Edition (2). ICD-O-2 codes that could not be unequivocally translated to a WHO diagnosis were categorized as lymphoma not otherwise specified (NOS). B-cell proliferations of uncertain malignant potential ( $n = 12$ ) were not considered in the present report, leaving 1,275 potentially eligible lymphoid tissue malignancies. After a few additional exclusions (82 prevalent tumors; 36 participants with a history of cancer; and 127 with insufficient serum samples), 1,030 lymphoid tissue malignancies (cases) were available for matching.

Two controls were selected for each case by incidence density sampling from all cohort participants who were alive and cancer-free at the time cases were diagnosed and for whom blood samples were available. They were matched with cases by center, sex, age ( $\pm 12$  months at blood collection), date ( $\pm 3$  months) and time of day at blood collection, and fasting status. No matched controls were available for 7 cases and only 1 control was available for 18 cases, thus leaving 1,023 cases and 2,028 controls. Cases eventually consisted of 628 B-cell NHLs [including 164 chronic lymphocytic leukemia (CLL)], 36 T-cell NHLs, 75 lymphoma NOS (collectively referred to as NHL,  $n = 739$ ), 238 MMs, and 46 HLs.

### Laboratory assays

Serologic assays were all conducted in the same laboratory (INSERM) using tests licensed in France after approval of analytic performance by a national regulatory authority. Cutoffs recommended by manufacturers were used to define positivity to each test.

Detection of antibodies against HCV (anti-HCV) was carried out on serum or plasma (for the Swedish centers), using a third-generation enzyme immunoassay (NANBASE C-96 3.0; General Biologicals Corporation; refs. 15, 16). Anti-HCV-seropositive samples were confirmed using a second enzyme immunoassay (HEPANOSTIKA HCV Ultra; Beijing United Biomedica Co. Ltd. ref. 17).

Antibodies against hepatitis B core antigen (anti-HBc, IgM, and IgG), a marker of past HBV exposure, were detected using an enzyme-linked fluorescent immunoassay based on an inhibition principle (VIDAS Anti-HBc Total II; BioMerieux; ref. 18). Testing for hepatitis B surface antigen (HBsAg), a marker of chronic carriage of HBV, was carried out using an enzyme-linked fluorescent immunoassay (VIDAS HBsAg Ultra; BioMerieux; ref. 19). HBsAg-seropositive samples were confirmed by a MicroElisa test (HEPANOSTIKA HBsAg Ultra; BioMerieux).

On account of the limited quantities of plasma available, testing for anti-HBc had to be omitted on plasma samples from Sweden. For the same reason, a different test for HBsAg had to be used on plasma samples from Sweden, that is, a solid-phase enzyme immunoassay [SURASE B-96 (TMB), General Biologicals Corporation].

### Statistical analysis

Conditional logistic regression, stratified by case-control set, was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) for NHL, MM, and HL by anti-HCV, anti-HBc, and HBsAg seropositivity. ORs were also calculated for the combination of the 3 malignancies overall and by time interval ( $<3$ ,  $\geq 3$  years) between blood sample collection and tumor diagnosis. The use of different time intervals (e.g.,  $<2$ ,  $\geq 2$ ; or  $<5$ ,  $\geq 5$  years) yielded similar results (data not shown).

### Results

Table 1 shows the mean age of participants at enrolment and hence at blood collection, the proportion of men and the number of NHL, MM, and HL cases, as well as the corresponding number of controls and their hepatitis viral marker status by country. The mean age at enrolment was 57.9 (5th–95th percentile: 43.5–70.8), but it varied by center. On account of the matching procedure, age and sex distribution was the same in cases and controls. Anti-HCV seropositivity ranged from 0% in Germany and Greece to 5.3% in Spain. Anti-HBc seropositivity was least frequent in Denmark (1.9%) and most frequent in Greece (45.9%). Finally, HBsAg seropositivity went from 0% in the Netherlands and the United Kingdom to 2.7% in Greece (Table 1). Only one individual (a control subject from Italy) was seropositive for both anti-HCV and HBsAg. Mean age at cancer diagnosis or reference date (among controls)

**Table 1.** Age at enrolment, sex, and hepatitis viral marker status of 1,023 cases of NHL, MM, and HL and 2,028 controls by country: EPIC cohort study

Country	Age, mean $\pm$ SD	Sex % men	NHL cases	MM cases	HL cases	Controls	Seropositivity among controls, %		
							Anti-HCV <sup>+</sup>	Anti-HBc <sup>+</sup>	HBsAg <sup>+</sup>
Denmark	57.7 $\pm$ 4.3	60.6	182	44	13	477	0.4	1.9	0.6
Germany	55.3 $\pm$ 8.0	62.3	73	26	7	212	0.0	7.1	0.5
Greece	62.5 $\pm$ 10.8	56.8	27	7	3	74	0.0	45.9	2.7
Italy	55.1 $\pm$ 8.1	35.7	78	21	5	207	3.9	21.8	2.4
Spain	55.3 $\pm$ 8.3	44.8	13	6	1	38	5.3	28.9	2.6
Sweden	57.9 $\pm$ 8.6	55.6	185	77	10	530	0.4	– <sup>a</sup>	0.8
The Netherlands	57.3 $\pm$ 7.0	15.0	60	19	1	160	1.3	2.5	0.0
United Kingdom	61.2 $\pm$ 8.7	51.5	121	38	6	330	0.6	3.3	0.0
<b>Total</b>	<b>57.9 <math>\pm</math> 7.9</b>	<b>51.4</b>	<b>739</b>	<b>238</b>	<b>46</b>	<b>2,028</b>	<b>0.9</b>	<b>8.6</b>	<b>0.8</b>

<sup>a</sup>Not tested because of insufficient amount of plasma.

**Table 2.** ORs and corresponding 95% CIs for NHL, MM, and HL by hepatitis viral marker status (1,023 cases and 2,028 controls): EPIC cohort study

Seropositivity	NHL		MM		HL	
	Ca:Co <sup>a</sup>	OR (95% CI) <sup>b</sup>	Ca:Co	OR (95% CI) <sup>b</sup>	Ca:Co	OR (95% CI) <sup>b</sup>
<b>Anti-HCV</b>						
No	730:1,454	1	237:467	1	44:89	1
Yes	9:14	1.30 (0.55–3.07)	1:3	0.55 (0.06–5.39)	2:1	4.00 (0.36–44.1)
<b>Anti-HBc<sup>c</sup></b>						
No	499:1,014	1	150:287	1	32:67	1
Yes	55:92	1.25 (0.86–1.84)	11:33	0.56 (0.25–1.25)	4:4	2.26 (0.49–10.4)
<b>HBsAg</b>						
No	728:1,456	1	232:467	1	45:89	1
Yes	11:12	1.78 (0.78–4.04)	6:3	4.00 (1.00–16.0)	1:1	2.00 (0.13–32.0)

Abbreviations: Ca: cases, Co: controls.

<sup>a</sup>One control with undetermined value for anti-HBc.

<sup>b</sup>Estimated from conditional logistic regression analysis, conditioned on center, sex, age, date and time of day at blood collection, and fasting status.

<sup>c</sup>Swedish samples not tested because of insufficient amount of plasma.

was 62.7 (5th–95th percentile: 48.8–75.5) but it varied by type of lymphoid malignancy (i.e., 62.9, 63.1, and 58.9 for NHL, MM, and HL, respectively; data not shown).

Table 2 shows the association between seropositivity to hepatitis viral markers and NHL, MM, and HL risk. The ORs associated with anti-HCV seropositivity were 1.30 (95% CI: 0.55–3.07), 0.55 (95% CI: 0.06–5.39), and 4.00 (95% CI: 0.36–44.1) for NHL, MM, and HL, respectively. The corresponding ORs by anti-HBc seropositivity were 1.25 (95% CI: 0.86–1.84), 0.56 (95% CI: 0.25–1.25), and 2.26 (95% CI: 0.49–10.4). Nonsignificant direct associations were found between seropositivity to HBsAg and the risk of NHL (OR = 1.78; 95% CI: 0.78–4.04) and HL (OR = 2.00; 95% CI: 0.13–32.0) whereas a statistically significant association was found with MM (OR = 4.00; 95% CI: 1.00–16.0 (Table 2)). When 164 CCL cases and their 326 matched controls were evaluated separately, no CLL and 2 controls were found to be seropositive to HBsAg. Subtraction of CLL from the NHL group led to an OR for seropositivity to HBsAg of 2.20 (95% CI: 0.93–5.18). Restriction to 690 B-cell lymphomas (i.e., NHL of B lineage and MM) and their corresponding 1,369 matched controls led to an OR of 2.11 (95% CI: 0.96–4.63) for seropositivity to HBsAg (data not shown).

The OR by seropositivity to HBsAg for the combination of the 3 malignancies was 2.21 (95% CI: 1.12–4.33) and did not differ by time interval between blood sample collection and tumor diagnosis. The OR was 2.22 (95% CI: 0.74–6.63) less than 3 years after and 2.20 (95% CI: 0.93–5.18) 3 years or more after blood collection (Table 3). There was no significant heterogeneity in the findings on HBsAg across countries ( $\chi^2$ , 6 degrees of freedom = 3.7;  $P = 0.717$ ). The association between the combination of lymphoid malignancies and seroposi-

tivity to HBsAg was also assessed separately in countries with high HBV prevalence (Greece, Italy, and Spain) and in low prevalence countries. Similar ORs (2.00; 95% CI: 0.75–5.33; and 2.41; 95% CI: 0.95–6.13, respectively) were found (data not shown).

## Discussion

The present study is the second largest population-based cohort study on the relationship between HCV or HBV infection and the risk of lymphoid malignancies (13). No clear association was found between

**Table 3.** ORs and corresponding 95% CIs for the combination of NHL, MM, and HL by HBsAg overall and by time interval between blood collection and tumor diagnosis (1,023 cases and 2,028 controls): EPIC cohort study

	HBsAg seropositivity		
	No Ca:Co	Yes Ca:Co	OR (95% CI) <sup>a</sup>
Overall	1,005:2,012	18:16	2.21 (1.12–4.33)
Interval, y			
<3	292:591	7:6	2.22 (0.74–6.63)
≥3	713:1,421	11:10	2.20 (0.93–5.18)

Abbreviations: Ca: cases, Co: controls.

<sup>a</sup>Reference category: HBsAg seronegative. Estimated from conditional logistic regression analysis, conditioned on center, sex, age, date and time of day at blood collection, and fasting status.

anti-HCV seropositivity and the risk of NHL, MM, or HL. Conversely, an association was discovered with HBsAg seropositivity that was statistically significant for MM and for the combination of the 3 malignancies.

The majority of the evidence regarding the role of HCV or HBV infection in the etiology of lymphoid tissue malignancies derives from case-control studies (9, 12). Except for a cohort study of more than 600,000 South Korean workers and their dependents, among whom 9% were HBsAg-seropositive but information on anti-HCV was not available (13), all prospective information on the topic derives from relatively small follow-up studies (20) or cohorts of individuals seropositive to anti-HCV (21, 22), HBsAg (23, 24), or both (25, 26). The pooled relative risk for NHL among anti-HCV-seropositive individuals in prospective studies was 2.03 (95% CI: 1.37–3.02) (9, 25). The range of ORs for NHL according to HBsAg seropositivity in 5 cohort studies from England and Wales (23), the United States (25), Taiwan (24), Australia (26), and South Korea (13) was similar (1.7–3.5) to that reported for anti-HCV seropositivity. With respect to MM, only one cohort study on HCV (OR = 2.42, 95% CI: 0.97–4.99; ref. 22) and one on HBV [hazard ratio (HR) = 0.90; 95% CI: 0.56–1.43; ref. 13] were available. No cohort studies reported data on the relationship between HL and anti-HCV, but the large cohort study from South Korea suggested no association between HBsAg seropositivity and HL (HR = 0.99; 95% CI: 0.48–2.04; ref. 13).

Our present findings on HBsAg seropositivity and the risk of NHL are, therefore, in agreement with the studies available on the topic, although the positive association in our study was not statistically significant. Conversely, an association between HBsAg seropositivity and MM risk has not been reported before, and the one between anti-HCV seropositivity and NHL and MM in the EPIC study was weaker than in most previous work (9), although not incompatible, on account of our broad CI ranges. Very few HL cases were available in the EPIC study and the disease was included for the sake of completeness.

The similarity between seropositivity to HBsAg (0.8%) and anti-HCV (0.9%) in EPIC participants was unexpected. The European Centre for Disease Prevention and Control reported that HCV infection has been the most common type of hepatitis in the European Union over the last decades, but surveillance data are severely incomplete (27). The non-negligible exposure to HBV infection among EPIC participants was confirmed by the 9% seroprevalence of anti-HBc, a marker of HBV exposure that was not, however, associated with the risk of NHL, MM or HL. The low HBsAg:anti-HBc seroprevalence ratio (1:11) suggested that in EPIC participants who had been infected with HBV the infection seldom became an active chronic infection. This finding would be consistent with acquisition of the infection in adulthood, possibly through sexual intercourse, rather than at birth or during childhood (28).

The hint of an association of predominately B-cell lymphoid tissue malignancies with active chronic

HBV infection, but not with past exposure, suggests that HBV might promote B-cell lymphomagenesis in a way similar to the mechanism proposed for HCV (i.e., chronic antigen stimulation of B-cells), possibly enhanced by engagement of B-cell surface receptors and costimulatory molecules (9). Both HCV and HBV can cause extrahepatic autoimmune diseases, such as cryoglobulinemia and immunocomplex deposition in polyarteritis nodosa, respectively (24, 29, 30). Both viruses are lymphotropic and can be detected in mononuclear cells (31) and the possibility of a direct oncogenic mechanism has been evaluated (32). Integration in the host cell genome, leading to a wide range of genetic alterations, has been shown for HBV but not HCV (32). Conversely, the reversibility of some virus-associated NHL after antiviral treatment has been reported for HCV but has never been evaluated for HBV-associated lymphoid tissue malignancies (29).

In agreement with the South Korean cohort (13), the association with HBsAg seropositivity in our study was not restricted to lymphoid malignancies that had arisen less than 3 years after blood collection, thus providing some reassurance against the possibility of reverse causation (i.e., reactivation of occult HBV infection due to immunologic alterations that precede tumor diagnosis). So far, reactivation of occult HBV infection has only been documented in anti-HBc-seropositive patients with hematologic malignancies undergoing chemotherapy (33).

The present study has strengths and weaknesses. It included nearly 1,000 lymphoid tissue malignancies, but anti-HCV and HBsAg seroprevalence was less than 1%. Different factors contributed to this low prevalence of hepatitis viruses. First, we had to exclude a substantial proportion of EPIC participants who were blood donors from Italy and Spain (i.e., the 2 countries in Europe where population-based prevalence of HCV infection is highest; refs. 34, 35). A "healthy volunteer" effect is also possible, as at least anti-HCV seropositivity was somewhat lower than that in the control groups of case-control studies on NHL carried out in some EPIC countries, such as Italy (34), Germany (36), Spain (35), and, to a lesser extent, Denmark and Sweden (37). Very high prevalence of HBV markers in Greece was expected, as the country had experience of a severe epidemic of HBV infection at least until the end of the 1970s (38).

In addition, serum samples have to be used very sparingly in long-term multihypothesis cohort studies such as EPIC. We were, therefore, unable to test for HCV RNA, HBV DNA, and HBeAg or the presence of other potentially relevant infections. Lack of information on HIV seropositivity is of special concern as HIV infection is associated with HCV and HBV infection and with increased risk of lymphoid malignancies (7). There are, however, good reasons to believe that the number of HIV-infected individuals among participants in the EPIC study was negligible. The earliest WHO estimates about HIV prevalence in Europe suggested that in 1998 there

were fewer than 10 HIV-infected persons per 100,000 population in all countries included in the present study and less than 3 per 100,000 in northern European countries (39). Most important, in the mid-1990s, the majority of HIV infections in Europe involved individuals (e.g., intravenous drug users) who were younger than the predominantly middle-aged participants in the EPIC study.

Finally, the low number of NHL associated with hepatitis viral markers prevented us from conducting separate analyses by histologic subtype. Morphologically, however, lymphomagenesis linked to chronic antigenic stimulation from HCV, and possibly HBV, can involve a variety of lymphoid tissue malignancies (9). A broad spectrum of histologic subtypes was indeed found among the 30 cases seropositive for anti-HCV or HBsAg (e.g., 5 diffuse large cell lymphomas, 4 follicular lymphomas, 2 CLLs, 1 extranodal marginal zone, and lymphoplasmacytic lymphoma in addition to 7 MMs, 7 lymphoma NOS, and 3 HLs).

In conclusion, the present nested case-control study from EPIC neither weakened nor strengthened the evidence of an association between HCV and NHL or other lymphoid tissue malignancies. However, it lent some additional support to the possibility that chronic HBV infection may have a role in lymphomagenesis. The proportion of lymphoid tissue malignancies attributable to HBV should be very low in European countries but can be high among HBsAg-seropositive individuals. In the large South Korean cohort study, for instance, the risk of NHL attributable to HBV among HBsAg-seropositive individuals was 43% (13). Treatment directed at control of HBV infection should, therefore, be evaluated in HBsAg-seropositive patients with lymphoid tissue malignancies.

In addition, the non-negligible prevalence of markers of HBV infection in healthy EPIC participants from countries where HBV vaccination of children or adolescents is not recommended (Denmark, the Netherlands,

and the United Kingdom; [www.who.int](http://www.who.int)) indirectly supports the WHO call for universal HBV vaccination even in low-endemicity countries (40).

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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### References

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010; 46:765–81.
2. Jaffe ES, Harris NL, Stein H, Vardiman J. World Health Organisation Classification of Tumours: Pathology and Genetics. Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.
3. Bosetti C, Levi F, Ferlay J, Lucchini F, Negri E, La Vecchia C. Incidence and mortality from non-Hodgkin lymphoma in Europe: the end of an epidemic? *Int J Cancer* 2008;123:1917–23.
4. Hirabayashi Y, Katanoda K. Comparison of time trends in multiple myeloma incidence (1973–1997) in East Asia, Europe and United States, from Cancer Incidence in Five Continents, Vols IV–VIII. *Jpn J Clin Oncol* 2008;38:720–1.
5. Adamson P, Bray F, Costantini AS, Tao MH, Weiderpass E, Roman E. Time trends in the registration of Hodgkin and non-Hodgkin lymphomas in Europe. *Eur J Cancer* 2007;43:391–401.
6. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. *Blood* 2006;107:265–76.
7. Pagano JS. Viruses and lymphomas. *N Engl J Med* 2002;347: 78–9.
8. Negri E, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004;111:1–8.
9. Dal Maso L, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2006;15:2078–85.
10. de Sanjosé S, Benavente Y, Vajdic CM, et al. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. *Clin Gastroenterol Hepatol* 2008;6:451–8.
11. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens—part B: biological agents. *Lancet Oncol* 2009;10:321–2.

12. Nath A, Agarwal R, Malhotra P, Varma S. Prevalence of hepatitis B virus infection in non-Hodgkin's lymphoma: a systematic review and meta-analysis. *Intern Med J* 2010;40:633–41.
13. Engels EA, Cho ER, Jee SH. Hepatitis B virus infection and risk of non-Hodgkin lymphoma in South Korea: a cohort study. *Lancet Oncol* 2010;11:827–34.
14. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
15. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trépo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J Viral Hepat* 2001;8:87–95.
16. Chevaliez S, Pawlotsky JM. Use of virologic assays in the diagnosis and management of hepatitis C virus infection. *Clin Liver Dis* 2005;9:371–82; v.
17. Fagundes GD, Bonazza V, Ceretta LB, Back AJ, Bettiol J. Detection of the hepatitis C virus in a population of adults. *Rev Lat Am Enfermagem* 2008;16:396–400.
18. Ollier L, Laffont C, Kechkekian A, Doglio A, Giordanengo V. Detection of antibodies to hepatitis B core antigen using the Abbott ARCHITECT anti-HBc assay: analysis of borderline reactive sera. *J Virol Methods* 2008;154:206–9.
19. Weber B, Van Der Taelen-Brulé N, Berger A, Simon F, Geudin M, Ritter J. Evaluation of a new automated assay for hepatitis B surface antigen (HBsAg) detection VIDAS HBsAg Ultra. *J Virol Methods* 2006;135:109–17.
20. Rabkin CS, Tess BH, Christianson RE, et al. Prospective study of hepatitis C viral infection as a risk factor for subsequent B-cell neoplasia. *Blood* 2002;99:4240–2.
21. Ohsawa M, Shingu N, Miwa H, et al. Risk of non-Hodgkin's lymphoma in patients with hepatitis C virus infection. *Int J Cancer* 1999;80:237–9.
22. Duberg AS, Nordstrom M, Torner A, et al. Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. *Hepatology* 2005;41:652–9.
23. Crook PD, Jones ME, Hall AJ. Mortality of hepatitis B surface antigen-positive blood donors in England and Wales. *Int J Epidemiol* 2003;32:118–24.
24. Fwu CW, Chien YC, Nelson KE, et al. Mortality after chronic hepatitis B virus infection: a linkage study involving 2 million parous women from Taiwan. *J Infect Dis* 2010;201:1016–23.
25. Ulcickas Yood M, Quesenberry CP Jr, Guo D, et al. Incidence of non-Hodgkin's lymphoma among individuals with chronic hepatitis B virus infection. *Hepatology* 2007;46:107–12.
26. Amin J, Law MG, Bartlett M, Kaldor JM, Dore GJ. Causes of death after diagnosis of hepatitis B or hepatitis C infection: a large community-based linkage study. *Lancet* 2006;368:938–45.
27. Rantala M, van de Laar MJ. Surveillance and epidemiology of hepatitis B and C in Europe—a review. *Euro Surveill* 2008;13:pii 18880.
28. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006;28:112–25.
29. Viswanatha DS, Dogan A. Hepatitis C virus and lymphoma. *J Clin Pathol* 2007;60:1378–83.
30. Trepo C, Guillevin L. Polyarteritis nodosa and extrahepatic manifestations of HBV infection: the case against autoimmune intervention in pathogenesis. *J Autoimmun* 2001;16:269–74.
31. Pasquinelli C, Laure F, Chatenoud L, et al. Hepatitis B virus DNA in mononuclear blood cells. A frequent event in hepatitis B surface antigen-positive and -negative patients with acute and chronic liver disease. *J Hepatol* 1986;3:95–103.
32. Tsai WL, Chung RT. Viral hepatocarcinogenesis. *Oncogene* 2010;29:2309–24.
33. Lalazar G, Rund D, Shouval D. Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies. *Br J Haematol* 2007;136:699–712.
34. Talamini R, Montella M, Crovatto M, et al. Non-Hodgkin's lymphoma and hepatitis C virus: a case-control study from northern and southern Italy. *Int J Cancer* 2004;110:380–5.
35. de Sanjose S, Nieters A, Goedert JJ, et al. Role of hepatitis C virus infection in malignant lymphoma in Spain. *Int J Cancer* 2004;111:81–5.
36. Nieters A, Kallinowski B, Brennan P, et al. Hepatitis C and risk of lymphoma: results of the European multicenter case-control study EPILYMPH. *Gastroenterology* 2006;131:1879–86.
37. Schollkopf C, Smedby KE, Hjalgrim H, et al. Hepatitis C infection and risk of malignant lymphoma. *Int J Cancer* 2008;122:1885–90.
38. Zacharakis G, Kotsiou S, Papoutselis M, et al. Changes in the epidemiology of hepatitis B virus infection following the implementation of immunisation programmes in northeastern Greece. *Euro Surveill* 2009;14:pii 19297.
39. World Health Organization, UNAIDS, EU Collaborating Centre on AIDS. HIV/AIDS surveillance in Europe. First half-yearly report 1999. Available from: [http://www.eurohiv.org/reports/report\\_61/pdf/report\\_eurohiv\\_61.pdf](http://www.eurohiv.org/reports/report_61/pdf/report_eurohiv_61.pdf); 1999.
40. Zuckerman J, van Hattum J, Cafferkey M, et al. Should hepatitis B vaccination be introduced into childhood immunisation programmes in northern Europe? *Lancet Infect Dis* 2007;7:410–9.