Correspondence: dr. Gisele Colleoni, Rua Botucatu, 740, 3º andar, Hematologia, CEP 043900-000, Vila Clementino, São Paulo, SP, Brazil. Fax: international +55.11.55718806.
E-mail: gcolleoni@hemato.epm.br

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Malignant Lymphomas

High incidences of malignant lymphoma in patients infected with hepatitis B or hepatitis C virus

It is still not clear whether there is an association between malignant lymphomas and hepatitis B or hepatitis C virus infections. Most studies from Italy and Japan support this association, but studies from other countries generally do not. We conducted a hospital-based case-control study to evaluate this association and show a significant association between the development of malignant lymphoma and infections by these viruses.

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There are conflicting data on an association between malignant lymphoma and hepatitis B virus (HBV) or hepatitis C virus (HCV) infections. Most studies from Italy[1,2] and Japan[3,4] support this association, but those from other countries[5,6] generally do not. We conducted a hospital-based case-control study in Japan to evaluate this association.

We selected 145 consecutive patients with malignant lymphoma who were admitted to Toranomon Hospital in Japan between 1995 and 2001. All the patients were first diagnosed with malignant lymphoma and had not received any chemotherapy. Controls were selected randomly from patients admitted to the orthopedics (n=290) and ear, nose and throat (ENT) (n=284) departments of the hospital. The case-patients were matched for age, sex and year of visit with the controls in a 1:2 ratio. We excluded control patients with a history of malignancy. Age was further adjusted as a continuous variable in conditional logistic regression analysis. The following data were extracted from the patients' medical records; age, sex, smoking habits, alcohol use, HBs antigen (HBs-Ag), anti-HCV antibody (HCV-Ab), anti-HTLV-1 antibody, anti-HIV antibody, and a history of malignancy. The definition of HBV and HCV infection was a positive result for HBs-Ag and HCV-Ab, respectively. The diagnoses of malignant lymphoma was confirmed by two hematopathologists who independently reviewed 126 available samples.

All statistical analyses were performed with STATA, ver.7 (software STATA Corp., College Station, TX, USA). Bivariate analyses were performed with the χ^2, Fisher's exact, or Wilcoxon signed-rank test. Attribution of HBV/HCV infection was evaluated in terms of odds ratios (OR) and 95% confidence intervals (CI). A conditional logistic regression model was applied to estimate ORs and 95% CI. Analyses were initially conducted separately for cases vs. orthopedic controls, and cases vs. ENT controls. However, since analyses for both control groups showed similar trends, these two subgroups of controls were combined and overall analysis was conducted. The *sampsi* procedure of STATA was used to calculate the statistical power necessary to detect a difference between observed percentages of subjects HBV/HCV infection among cases and controls with a type I error of 0.05. Estimated values were 60% for HBV in two-tailed analysis, when the prevalence of infection was 5% among cases and 1% among controls. The corresponding value for HCV was 55%, when its prevalence was 11% among cases and 5% among controls. The study protocol was approved by the institutional review board.

The patients' characteristics are shown in Table 1. Of the 145 cases, 120, 16, and 7 were diagnosed with B-cell, T/NK-cell neoplasms, and Hodgkin's lymphoma, respectively. Two other patients had malignant lymphoma but the histologic subtype could not be classified. Seven patients with HBV infection developed malignant lymphoma: the histologic subtypes were diffuse large B-cell (n=4), follicular B-cell (n=1), and adult T-cell lymphoma (n=1). The prevalence of HBV infection among cases was significantly higher than among orthopedic (OR: 4.67, $p = 0.026$), ENT (OR: 4.67, $p = 0.01$) or combined (OR: 4.67, $p = 0.006$) controls. Age-adjusted OR (aOR) between cases and orthopedic (aOR: 4.74, $p = 0.024$), ENT (aOR: 8.34, $p = 0.006$) or combined (aOR: 5.36, $p = 0.003$) controls were significant. Fifteen patients with HCV infection developed malignant lymphoma. Histologic subtypes were diffuse large B-cell (n=11), follicular B-cell (n=2), peripheral T-cell (n=1), and unclassified (n=1). The prevalence of HCV infection among cases was higher than among orthopedic (OR:3.83, $p = 0.002$), ENT (OR:2.06, $p=0.099$) or combined (OR:1.96, $p = 0.005$) controls. The aOR between cases and orthopedic (aOR:3.80, $p = 0.002$), ENT (aOR:1.86, $p = 0.086$) and combined (aOR:2.17, $p = 0.002$) controls were significant. Multivariate analysis showed significant associations between both HBV and HCV infection, and malignant lymphoma (Table 2).

Considering the incidence of malignant lymphoma, case-control studies are the most feasible method to evaluate associations between HBV/HCV and lymphoma. However, most previous case-control studies had some methodological limi-

Table 1. Patients' characteristics.

	Case group (Lymphoma group) n=145	*Control group 1 (Orthopedic group) n=290*	*Control group 2 (Otolaryngology group) n=284*	*Total controls n=574*
Female: Male	57:88	114:176	109:175	223:351
Age (range, median), y	46-94, 64	44-95, 63	44-91, 62	44-95, 63
Subjects by age group; n(%)				
40-49 (%)	14 (9.7)	24 (8.2)	28 (9.9)	52 (9.1)
50-59 (%)	41 (28.3)	94 (32.9)	102 (35.9)	196 (34.1)
60-69 (%)	39 (26.9)	69 (23.6)	64 (22.5)	133 (23.2)
70-95 (%)	51 (35.2)	103 (35.3)	90 (31.7)	193 (33.6)
HBs-Ag: n(%)				
Positive	7 (4.8)	3 (1.0)	3 (1.1)	6 (1.0)
Negative	132 (91.0)	283 (97.6)	279 (98.2)	562 (97.9)
Unknown	6 (4.1)	4 (1.4)	2 (0.7)	6 (1.0)
HCV-Ab: n(%)				
Positive	16 (11.0)	9 (3.1)	20 (7.0)	29 (5.1)
Negative	124 (85.5)	277 (95.5)	262 (92.3)	539 (93.9)
Unknown	5 (3.4)	4 (1.4)	2 (0.7)	6 (1.0)
HBs-Ag and HCV-Ab Positive	0 (0)	0 (0)	1 (0.4)	1(0.2)
HBs-Ag or HCV-Ab Positive	23 (15.9)	12 (4.1)	21 (7.4)	33 (5.7)
HBs-Ag and HCV-Ab Negative	117 (80.7)	274 (94.5)	260 (91.5)	534 (93.0)
Unknown*	5 (3.4)	4 (1.4)	2 (0.7)	6 (1.0)

**Subjects with either of HBV or HCV status was unknown*

Table 2. Age adjusted Odds Ratios and 95% Confidence Intervals of HBV and HCV Infection for Lymphomas by Multivariate Analysis*1.

Cases vs.	*Control Group1 (Orthopedics)*	*Control Group 2 (Otolaryngology)*	*Total Controls*1*
HBV			
Adjusted OR	5,22	7,78	5,21
95% CI	1.32-20.6	1.72-35.3	1.71-15.8
p-value	0,018	0,008	0,004
HCV			
Adjusted OR	3,73	1,95	2,45
95% CI	1.56-8.91	0.77-4.92	1.23-4.88
p-value	0,003	0,158	0,011

**1 Age, HBV and HCV infectious status were included in multivariate analysis. *2 Twelve subjects were excluded from analysis because at least one of these variables was unknown.*

tations. First of all, some studies did not have enough power to detect the differences between case and controls.[7] Secondly, although matching is important to eliminate random error, it was not mentioned whether this was done in some studies.[5] We created two independent control groups in order to minimize random error. Lastly, some studies used potentially unsuitable controls such as healthy blood donors. Because individuals at risk of harboring blood-born pathogens are discouraged from donating blood, the prevalence of HBV/HCV infections among blood donors probably underestimates the prevalence in the general population. A few studies, including ours, have circumvented these problems. All these studies showed a significant association between HBV/HCV infection and lymphoma.[2,8,9]

The results of our study can be interpreted in two ways. Firstly, HBV/HCV infections might have contributed to lymphomagenesis. Neither HCV nor HBV sequences can be integrated into the host genome; they do, however, appear to act as an exogenous stimulus, triggering clonal B-cell expansion. The report that low-grade lymphoma has been eradicated following interferon-α treatment supports the theory of causality.[10] The second interpretation is the presence of an unrecognized bias, since two genetically different viruses without any similarity to oncogene sequences are associated with lymphomagenesis. The immunodeficiency predisposing patients to HBV/HCV infections might play a role in lymphomagenesis. Since the present study was hospital-based, caution is required in generalizing the results. Further studies are still required to clarify the association between HBV/HCV infections and malignant lymphomas.

*Hiroshi Iwata,[*α] Keitaro Matsuo,[°α] Kengo Takeuchi,[#] Yukiko Kishi,[@] Naoko Murashige,[@] Masahiro Kami[@]*

[*]Department of Hematology, Toranomon Hospital, Tokyo, Japan; [°]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [#]Department of Pathology, the University of Tokyo Hospital, Tokyo, Japan; [@]Hematopoietic Stem-cell Transplantation Unit, the National Cancer Center Hospital, Tokyo, Japan.

[α]These authors contributed equally to this work.

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Correspondence: Masahiro Kami, M.D., Hematopoietic Stem Cell Transplant Unit, the National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Phone: international +81.3.35422511. Fax: international +81.3.35423815. E-mail: mkami@ncc.go.jp

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Malignant Lymphomas

Soluble syndecan-1 levels in different plasma cell dyscrasias and in different stages of multiple myeloma

We measured serum levels of syndecan-1 in patients with multiple myeloma (MM), solitary plasmocytoma and monoclonal gammopathy of undetermined significance (MGUS). We then studied serum syndecan-1 levels in MM patients stratified by the Durie-Salmon staging system and the correlation of syndecan-1 levels with well known independent prognostic factors of MM.

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We investigated 67 patients: 13 had monoclonal gammopathy of undetermined significance (MGUS), 4 had solitary plasmocytoma, and 50 had multiple myeloma (MM). All plasma cell dyscrasias were defined by the diagnostic criteria of the American Southwest Oncology Group (SWOG).[1] For comparative analysis of patients the following data and parameters were registered: age, sex, percentage of plasma cells in the bone marrow, immunoglobulin (Ig) class, serum M-protein concentration, serum levels of β2-microglobulin, albumin, calcium, creatinine, total alkaline phosphatase, and C-reactive protein (CRP).

Patients were subdivided by the Durie-Salmon staging system.[2] At the time of evaluation 35 patients with MM had already undergone some form of chemotherapy. The other group of 15 previously untreated MM patients were followed during their chemotherapy. The median follow-up period of the patients was 6 months. Seven patients received ICOMP (idarubicin, cyclophosphamide, vincristine, methylprednisone) therapy, and 8 patients received VAD (vincristine, doxorubicin, dexamethasone) therapy for 6 months. All patients were co-treated with bisphosphonates. Response to therapy was determined by the change in M-protein concentration and the ratio of bone marrow plasma cells 6 months after initiation of chemotherapy. Objective therapeutic response was defined as at least a 50% reduction of serum paraprotein concentration.

The serum concentration of soluble syndecan-1 was measured using a commercially available human syndecan-1 enzyme-linked immunosorbent assay (ELISA) kit (Diaclone Research, Besancon, France).

Results were considered statistically significant when the *p* value was less than 0.05. Comparisons between groups were determined with the Mann-Whitney U-test. Response to treatment was analyzed with multiple logistic regression techniques.

Serum concentrations of syndecan-1 and correlation with other prognostic factors of MM. The median serum syndecan-1 concentrations of patients with MGUS (n=13) and solitary plasmocytoma (n=4) were 77.9 ng/mL (range: 33-122) and 65.6 ng/mL (range: 33.8-94.5), respectively. In contrast, the median syndecan-1 concentration in the group of MM patients (n=50) was 223.8 ng/mL (range: 36-508). Although the numbers of patients with MGUS and solitary plasmocytoma group were low, differences between the median syndecan-1 values were statistically significant.

Table 1. Correlations between serum syndecan-1 and other variables.

	Serum creatinine	*Serum β2-microglobulin*	*Plasma cell content (%) of bone marrow*	*Serum M-component*
N	50	49	49	49
r	0.378	0.379	0.407	0.558
p	0.007	0.007	0.004	<0.001

r: indicates correlation coefficient for syndecan-1 and the designated variable.

Statistically significant differences were also observed in median syndecan-1 levels in the MM group stratified according to Durie-Salmon stage (values given in ng/mL, number of patients and range in parentheses): group I/A: 61 (n=4, 36-102), II/A: 188.6 (n=20, 65.3-344), II/B: 260.6 (n=3, 208-267), III/A: 300.2 (n=12, 157-466), and III/B: 253.5 (n=11, 97.3-508). As shown in Table 1 a clear correlation was found with other well known prognostic factors of MM such as serum creatinine, β_2-microglobulin, monoclonal protein concentration, and bone marrow plasma cell content.

Follow up of MGUS and MM patients. Over a 6-month follow-up period there was no increase in serum syndecan-1 level in the MGUS patients (n=13). Baseline serum syndecan-1 levels were determined before initiation of chemotherapy, whereas follow-up syndecan-1 levels were measured once after 6 months of treatment in the MM patients followed sequentially (=15). As shown in Table 2, there was a significant decrease in the median syndecan-1 level among patients who responded to chemotherapy. In contrast, no change was observed in patients who did not respond to chemotherapy. Syndecans are a family of cell surface proteoglycans.[3] The molecule is expressed on mature plasma cells.[4] Syndecan-1 mediates specific adhesion of myeloma cells to type I collagen and also mediates cell-cell adhesion between myeloma cells.[5-8]

Measured by a semiquantitative method, syndecan-1 levels were elevated in the sera of 7 out of 20 myeloma patients. Higher serum levels of soluble syndecan-1 were associated with higher levels of serum β_2-microglobulin and elevated plasma cell content in the bone marrow.[9] Evaluation of data collected from 138 MM patients showed that the serum syndecan-1 concentration could serve as an independent prognostic parameter in addition to serum β_2-microglobulin and WHO performance status.[10] In our study, patients with MM showed a higher median level of serum syndecan-1 than did patients with MGUS or plasmocytoma. The differences between these groups were significant.

A comparison of serum syndecan-1 levels among the myeloma subgroups also revealed significant differences. A