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Annals of Oncology 21: 1163–1167, 2010
doi:10.1093/annonc/mdp583
Published online 17 December 2009

Bone metastasis and poor performance status are prognostic factors for survival of carcinoma of unknown primary site in patients treated with systematic chemotherapy

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Received 18 May 2009; revised 30 August 2009; accepted 12 October 2009

Background: Cancer of unknown primary site (CUP) generally has a poor prognosis, and there is no established standard therapy. There have been no reports of a prognostic model for CUP patients treated with a single regimen of systemic chemotherapy.

Methods: Univariate and multivariate prognostic factor analysis for overall survival (OS) were conducted retrospectively in 58 consecutive CUP patients treated with carboplatin plus paclitaxel (Taxol) therapy as a first-line treatment.

Results: Univariate prognostic factor analysis revealed baseline performance status (PS) of two or more, low serum albumin level, pleural effusion, bone metastasis, and liver metastasis as adverse prognostic factors. Cox proportional hazards analysis showed that poor PS and bone metastasis had the most powerful adverse impact on survival. We developed a prognostic model using those two variables—a good-risk group (PS 0–1 without bone metastasis) and a poor-risk group (PS ≥2 or bone metastasis). The poor-risk group showed significantly poorer OS than the good-risk group (1 year OS 36.8% versus 67.1%, $P = 0.0003$).

Conclusions: Poor PS and bone metastasis were identified as independent adverse prognostic factors in CUP. A simple prognostic model was developed and seems useful for decision making as to whether chemotherapy is indicated for CUP patients.

Key words: cancer of unknown primary site, carboplatin plus paclitaxel, bone metastasis

introduction

Cancer of unknown primary site (CUP) is pathologically diagnosed metastatic carcinoma in which no obvious primary site is identified with a conventional work-up. It is not a rare clinical entity, accounting for 3%–5% of all solid malignancies [1, 2]. The prognosis of CUP is generally considered poor, with median survival ~6–12 months [3]. Briasoulis et al. [4] reported encouraging results from phase II data of carboplatin and paclitaxel combination therapy for patients with CUP. In this study, the overall response rate by an intention-to-treat analysis was 38.7%, and median overall survival (OS) was 13 months at median follow-up time of 28 months. Platinum and taxane combination therapy is now widely used in clinical practice [4–8], but recent multiple-treatment meta-analysis showed that no type of chemotherapy has been proven to prolong survival in patients with CUP [9]. CUP consists of heterogeneous neoplasms with variable biological features, making it difficult to identify clinically useful prognostic survival factors. But several subsets have been identified requiring a specific treatment and having a better prognosis. Women with peritoneal carcinomatosis of serous adenocarcinoma [10], women with adenocarcinoma of axillary lymph nodes [11] or cervical lymph node metastasis of squamous cell carcinoma [12], young adults with poorly differentiated carcinoma of midline distribution [13], and undifferentiated carcinoma with neuroendocrine features [14] are CUP subgroups known to have a better prognosis. But the majority of CUPs have a poor prognosis, as mentioned above. In this article, we report the results of a prognostic factor analysis conducted in a population of 58 patients of CUP treated with carboplatin and paclitaxel as a first-line systemic chemotherapy. We retrospectively investigated baseline characteristics as prognostic factors for survival to identify a subset of patients who would benefit from chemotherapy.

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methods

patient characteristics

The medical and pathological records of 58 consecutive newly diagnosed patients with CUP who received carboplatin and paclitaxel (Taxol, Bristol-Myers Squibb, Tokyo, Japan) combination therapy as first-line therapy at the Cancer Institute Hospital, Japanese Foundation for Cancer Research, from March 2004 to January 2008 were retrospectively reviewed. Patients had pathologically confirmed metastatic cancer and were surveyed for detailed medical history, complete physical examination, blood counts, chemistry profile, chest radiograph, computed tomography (CT) scan of chest and abdomen, and further radiological survey or endoscopy of suspected areas. Serum prostate-specific antigen (PSA) was measured in male patients, and CA 125 was measured in female patients. Women with adenocarcinoma of axillary lymph nodes also received mammography and breast ultrasound. Young adults with poorly differentiated adenocarcinoma involving the mediastinal region were surveyed with α-fetoprotein and β-human chorionic gonadotropin. The gastrointestinal tracts of male and female patients with adenocarcinoma involving abdominal and pelvic lesion were surveyed by upper gastrointestinal endoscopy and colonoscopy. Gynecologic examination was carried out in female patients with abdominal and pelvic disease. Patients with squamous cell carcinoma of cervical lymph nodes also underwent laryngeal endoscopy and upper gastrointestinal endoscopy. Bone metastases were assessed by the combination of bone scintigraphy or positron emission tomography with chest X-ray, CT, or magnetic resonance imaging. Histopathological review including immunohistochemistry (IHC) was carried out to detect primary sites and to exclude other malignancies. Low-molecular cytokeratins (CKs) 7 and 20 were routinely stained for all patients with CUP, and thyroid transcription factor 1, caudal type homeobox transcription factor 2, and PSA were stained for patients with adenocarcinoma of CUP. When a specific origin was suspected by morphological examination and clinical history, distinctive IHC was carried out (chromogranin, synaptophysin, and CD56 for neuroendocrine cell carcinoma; D2-40, placental alkaline phosphatase, human chorionic gonadotropin, and CD30 for germ-cell tumor; and D2-40 and calretinin for mesothelioma). In the case of difficulty in diagnosing epithelial carcinoma, several IHC of S100, vimentin, leukocyte common antigen, and CKs are used for distinguishing melanoma, sarcoma, and lymphoma from the anaplastic cell type of carcinoma.

We excluded patients in favorable subsets that have specific treatments other than carboplatin and paclitaxel—such as women with adenocarcinoma of axillary lymph nodes or cervical lymph node metastasis of squamous cell carcinoma, young adults with poorly differentiated carcinomas of midline distribution, and patients with undifferentiated carcinomas of neuroendocrine features. However, women with peritoneal carcinomatosis of adenocarcinoma who were treated with carboplatin and paclitaxel as first-line treatment were included in this study.

treatment

Carboplatin was administered by a 2-h i.v. infusion, dosed with 6 mg/ml/min target area under the free carboplatin plasma concentration versus time curve and was followed by paclitaxel 200 mg/m^2 in 500 ml of normal saline administered over 3 h. The Calvert formula was used for carboplatin dosing, on the basis of a glomerular filtration rate calculated by the Cockcroft–Gault equation using serum creatinine, body surface area, and age. Chemotherapy cycles were repeated every 3 weeks and responding patients continued the chemotherapy until disease progression or intolerable toxicity. Response to chemotherapy was assessed by Response Evaluation Criteria In Solid Tumors (RECIST, version 1.0). Progression-free survival (PFS) and OS were calculated from day 1 of the first cycle of chemotherapy.

statistical analysis

Survival curves were estimated using the Kaplan and Meier method, compared using the log-rank test, and prognostic factors were identified by univariate analysis. Then the forward stepwise Cox proportional hazards analysis was carried out to identify independent prognostic factors. Statistical analyses were carried out using SPSS software (version 17.0; SPSS Inc., Chicago, IL).

results

patient characteristics

Patient characteristics are shown in Table 1. Fifty-eight CUP patients treated with at least one cycle of carboplatin and paclitaxel combination therapy were retrospectively analyzed. Twenty-eight (48.3%) patients were male, and the median age was 64 years (range 28–79 years). Forty-nine patients (84.5%) had a good performance status (PS) of zero to one. Twenty-six (44.8%) patients had well-differentiated adenocarcinoma, 21 (36.2%) patients had anaplastic or poorly differentiated carcinoma, and 5 patients (8.6%) had squamous cell carcinoma. Another six (10.3%) patients had clear-cell carcinoma, transitional cell carcinoma, or adenosquamous cell carcinoma. Metastatic sites are listed in Table 1. Lymph nodes, lung, bone, and liver were frequently involved sites and cervical, mediastinum, and retroperitonium were common sites for lymph node metastasis.

PSA was measured in 20 male patients (median PSA level 2.04 ng/ml, range 0.34–4.04 ng/ml), and CA 125 was obtained in 26 female patients (median CA 125 level 462 U/ml, range 4.8–50000 U/ml). Five of six male patients with bone metastasis showed a PSA level <4.0 ng/ml, and the PSA value before treatment of one young male patient was not available.

outcome of chemotherapy

A total of 315 cycles were administered, and patients received a median of five cycles of treatment (range 1–21 cycles).

Table 1. Patient characteristics

Number of patients	58
Age, median (range)	64 (28–79)
Sex	
Male	28
Female	30
Performance status	
0–1	49
2–4	9
Pathology	
Adenocarcinoma	26
Squamous cell carcinoma	5
Poorly differentiated/anaplastic carcinoma	21
Other	6
Sites of metastasis	
Lung	15
Bone	13
Liver	11
Pleural effusion	15
Ascites	11
Lymph node	44

The response rates by main histopathological types of adenocarcinoma, squamous cell carcinoma, poorly differentiated carcinoma, or poorly differentiated adenocarcinoma were 42.3%, 60.0%, and 23.8%, respectively (Table 2). For other histology types, one patient with transitional cell carcinoma had partial response. Sixteen patients were treated with second-line chemotherapy. At a median follow-up time of 12 months (range 6–1659 days), median OS and PFS were 16.7 months and 5.9 months, respectively. Six patients had PFS >2 years and one of these patients survived >4 years.

prognostic model of clinical and biological variables

The outcome of univariate analysis of clinical and biological factors is listed in Table 3. Five parameters have prognostic relevance: poor PS (≥2) ($P = 0.01$), low serum albumin level (<3.7 g/dl) ($P = 0.003$), pleural effusion ($P = 0.04$), bone metastasis ($P = 0.02$), and liver metastasis ($P = 0.02$). Multivariate analysis for these five variables was conducted and showed that bone metastasis ($P = 0.002$) and PS of two or more ($P = 0.016$) had significant adverse impact for survival (Table 3). Poor PS was not correlated with presence of bone metastasis.

Table 2. Treatment results

	N	CR (*n*)	PR (*n*)	ORR (%)
Total	58	5	15	34.5
Pathology				
Adenocarcinoma	26	5	6	42.3
Squamous cell carcinoma	6	0	3	50.0
Poorly differentiated anaplastic carcinoma	21	0	5	23.8

CR, complete response; PR, partial response; ORR; overall response rate.

Table 3. Univariate and multivariate analysis of prognostic factors for survival

	Univariate	Multivariate	
	P value	HR (95% CI)	*P* value
PS ≥2	0.01	2.93 (1.22–7.04)	0.016
Age (>65 years)	0.29		
Sex (male)	0.41		
ALP (>UNL)	0.13		
LDH (>UNL)	0.45		
ALB (<3.7 g/dl)	0.003		
Hb (<11.0 g/dl)	0.77		
Pleural effusion	0.04		
Ascites	0.69		
Lung metastasis	0.58		
Bone metastasis	0.02	3.48 (1.56–7.78)	0.002
Liver metastasis	0.02		
Adenocarcinoma	0.81		
Poorly/anaplastic carcinoma	0.32		

HR, hazard ratio; CI, confidence interval; PS, performance status; ALP, alkaline phosphatase; UNL, upper normal limit; LDH, lactate dehydrogenase; ALB, albumin; Hb, hemoglobin.

The incidence of bone metastasis was not significantly different between males and females (6 of 28 males, 5 of 30 females). A prognostic model was developed with those two variables. Nineteen (32.8%) patients were assigned to the good-risk group (defined as PS 0–1 without bone metastasis), and 38 (67.2%) patients were assigned to the poor-risk group (defined as PS ≥2 or bone metastasis). The poor-risk group ($n = 19$) showed significantly poorer OS than good-risk group ($n = 39$) (1 year OS 36.8% versus 67.1%, $P = 0.0003$) (Figure 1).

discussion

To identify a favorable or poor prognostic group of patients with CUP is of great concern when physicians consider whether systemic chemotherapy is indicated. No randomized trial showed better survival with chemotherapy than best supportive care. To our knowledge, the current study is the first that assesses prognostic factors for survival of patients with CUP treated with a single first-line regimen and should give us information as we choose an optimal therapy.

We demonstrated an overall response rate of 34.5% and a median OS of 16.7 months in CUP patients by an intention-to-treat analysis. This result seems similar to the results previously reported by Briasoulis et al. [4] and slightly better than other reports. One reason might be that both studies included female patients with peritoneal carcinomatosis (11 of 58 in ours and 19 of 75 in Briasoulis et al.). In our study, seven women (63.6%) responded to chemotherapy. A second reason might be that our group included a marginally larger number of patients with good PS. Golfinopoulos et al. [9] reported in recent multiple-treatment meta-analysis for CUP that 10 randomized trials assessed in that study included variable rates for patients with poor PS (median 24.5%, interquartile range 12.8%–38.9%). Third, our study included a slightly smaller number of patients

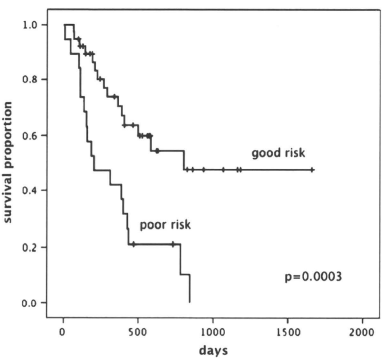

Figure 1. The prognostic model incorporating two variables. The good-risk group ($n = 39$) was defined as performance status (PS) of zero to one without bone metastasis and the poor-risk ($n = 19$) group as PS of two or more, or bone metastasis.

with liver metastasis, which was reported as an independent poor prognostic factor by Seve et al. [15]. But the rates of liver metastases in previous studies are variable from 16% to 76% [4–8, 16, 17]. The patients in the present study were treatable with combination therapy, so most of them maintained good PS and end organ function. No prospective studies or meta-analysis of prognostic factors for CUP have been published. But several retrospective studies have shown a number of independent adverse factors such as age, male gender, poor PS, adenocarcinoma histology, number of metastatic sites, liver metastasis, bone metastasis, lung metastasis, pleural metastasis, brain metastasis, comorbidity scoring of adult comorbidity evaluation-27 (ACE-27), low serum albumin, high serum lactate dehydrogenase (LDH), high serum alkaline phosphatase, lymphopenia, anemia, thrombocytopenia, high serum carcinoembryonic antigen, and high serum CA 125 [15, 18–20]. Abbruzzese et al. [18] reported adverse prognostic variables from a study of 657 cases of CUP at M. D. Anderson Cancer Center, and multivariate analysis identified male gender, a large number of metastatic sites, adenocarcinomatous histological type, and the presence of liver metastasis as unfavorable indicators. Culine et al. [19] proposed a simple prognostic model using PS and serum LDH levels in a population of 150 CUP patients, excluding favorable subsets, at a French cancer center. More recently, Seve et al. conducted a retrospective study assessing the influence of comorbidities, age, PS, and chemotherapy on survival in a population of 389 patients with CUP in Canada. Multivariate analysis showed that patients who had a PS of two or more and a high overall ACE-27 score had a poor prognosis. They concluded that the impact of comorbidity on survival was limited to patients with low PS [20]. The same author showed in another study that low serum albumin level and liver metastasis were the two most powerful adverse prognostic factors. The prognostic significance of those two factors was validated in another set of 124 patients with CUP [15]. In our study, bone metastases and poor PS (≥2) had a powerful adverse impact on survival. In clinical practice, bone metastases could be the cause of declining PS, but in this study, bone metastases and poor PS were not significantly correlated. Poor PS was also an adverse prognostic factor in studies by Culine et al. and by Seve et al. Bone metastases have been identified as an independent poor prognostic factor for the first time in uniformly treated patients with CUP. Prognostic significance of bone metastases in advanced cancer depends on the primary sites. In breast cancer or prostate cancer, the presence of bone metastases or bone-only metastases indicates a better prognosis [21]. On the other hand, the presence of bone metastases indicates a worse prognosis in lung cancer [22], thyroid cancer [23], or renal cell carcinoma [24]. The worse prognosis of patients with bone metastases in our series might be due to the apparent absence of occult breast cancer or prostate cancer in this set of patients.

Although our study might be small for finding independent prognostic factors retrospectively, it is important to identify clinically useful prognostic factors for CUP patients treated with platinum and taxane combination therapy, which are used frequently in daily practice. It has not been proven that systemic chemotherapy would prolong the survival of unfavorable CUP patients, and the best supportive care is a reasonable choice for patients who have little benefit from systemic chemotherapy.

We designed a new prognostic model that incorporated those two factors, poor PS and bone metastasis. The OS of patients with at least one or more prognostic factor was significantly shorter than those with no adverse prognostic factor. This model might be useful for decision making regarding the use of chemotherapy for CUP patients in daily clinical practice. A validation study of our prognostic model is warranted in the near future.

funding

A Health and Labour Sciences Research grant from the Ministry of Health, Labour and Welfare of Japan (2007-Clinical Cancer Research-029).

acknowledgements

The authors are grateful to staff members including Sayuri Nishiayma, Eriko Nara, Hiroaki Asai, Atsushi Katsube, and Hiroaki Goto for treating the patients at the Cancer Institute Hospital. Author contributions: MK designed the study, treated the patients, collected clinical data, and wrote the paper; SY and KU treated the patients and collected clinical data; KT, NY and YI diagnosed pathological specimens; YM, MY and YT treated the patients and assisting with writing the paper; KH designed the study and supervised the writing of the paper; ST treated the patients and designed the study and supervised all aspects of the research and analysis and contributed to the paper.

disclosure

The funding was coordinated by K. Nakagawa, Kinki University, Osaka, Japan.

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original article

Annals of Oncology 21: 1217–1221, 2010
doi:10.1093/annonc/mdp490
Published online 2 November 2009

Statin-independent prognosis of patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy

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Received 6 July 2009; revised 9 September 2009; accepted 10 September 2009

Background: A recent laboratory study indicated that statins impaired the antitumor effects of rituximab by inducing conformational changes in CD20. Although these findings raised significant concerns about statin use during rituximab treatment, their clinical significance is unclear.

Patients and methods: We conducted a retrospective study investigating the effects of statins on the prognosis of diffuse large B-cell lymphoma (DLBCL) treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP). Newly diagnosed DLBCL patients were analyzed ($n = 256$), including 35 patients taking statins.

Results: The 3-year progression-free survival rates were 84% and 73% ($P = 0.38$), while the overall survival rates were 89% and 78% ($P = 0.28$) for those patients treated with and without statins, respectively. After adjusting for the International Prognostic Index and serum cholesterol level, statin use was not associated with prognosis.

Conclusions: These results indicate that statins do not influence the clinical prognosis of DLBCL treated with RCHOP. Further studies with larger numbers of patients are warranted to confirm the prognostic significance of statins for patients with DLBCL receiving rituximab-containing chemotherapy.

Key words: diffuse large B-cell lymphoma, rituximab, statin

introduction

Rituximab, a chimeric anti-CD20 antibody, is highly effective in the treatment of various types of CD20-positive B-cell lymphomas [1, 2]. It has been reported that complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and the induction of apoptosis are the major mechanisms of action for rituximab [3, 4]. A significantly improved outcome has been obtained in young and elderly patients by combining cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) with rituximab [5–7].

3-Hydroxy-3-methylgutaryl coenzyme A reductase inhibitors, commonly known as statins, inhibit the rate-limiting step of the mevalonate pathway, which is essential for the biosynthesis of various compounds, including cholesterol [8]. The activity of rituximab may be reduced by conformational changes in CD20 induced by the depletion of serum cholesterol, which impairs rituximab binding and thereby significantly decreases rituximab-mediated CDC and ADCC against B-cell lymphoma cells [9].

Several authors have cited the same laboratory study [10–12] and these *in vitro* data have led some to recommend that statins might not be used when patients are being treated with rituximab. However, it is unclear whether statins actually affect the prognosis of diffuse large B-cell lymphoma (DLBCL) patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP). The aim of this study was to assess the influence of statin use on the outcome of patients with RCHOP-treated DLBCL.

patients and methods

patient characteristics

We reviewed the medical records of patients with DLBCL who received RCHOP as a first-line therapy at the Cancer Institute Hospital and Okayama University Hospital between April of 2004 and May of 2008 and were followed up until January of 2009. In this period, the patients with

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statin use and the patients without statin use treated in the same time frame as the patients with statin-use group were analyzed. The definition of the patients with statin use was the patients who had been taking statins for at least 2 months at the start of RCHOP. The study protocol for this retrospective analysis was approved by the institutional review boards of both hospitals. Patients were analyzed if they were older than 18 years and had a performance status (PS) of zero to three according to the criteria of the Eastern Cooperative Oncology Group. Patients were excluded if they were positive for antibodies against human immunodeficiency virus-1 or -2. Patients with primary mediastinal large B-cell lymphoma, primary central nervous system lymphoma, or primary testicular lymphoma were also excluded from this study.

The disease stage was evaluated according to the Ann Arbor staging system. All patients had undergone staging investigations, including physical examinations, blood and serum analyses, bone marrow aspiration and biopsy, and computed tomography (CT) of the neck, chest, abdomen, and pelvis. Magnetic resonance imaging was used to evaluate involved organs in the head and neck. Some patients underwent positron emission tomography (PET)/CT with 2-[fluorine-18]fluoro-2-deoxy-D-glucose for staging evaluation and assessing treatment response. Chemotherapy sensitivity was defined according to standard volume criteria using CT and PET/CT imaging [13, 14].

The following clinical and laboratory information was available at the time of diagnosis: history of statin treatment; age; sex; PS; stage; number of extranodal sites; and serum levels of lactate dehydrogenase, total cholesterol (T-cho), triglycerides, and high-density lipoprotein cholesterol. This information allowed the determination of International Prognostic Index (IPI) scores for the included patients. Patients were categorized into either low-risk (IPI score 0–2) or high-risk (IPI score 3–5) groups. The serum cholesterol level was considered 'high' when higher than the median serum T-cho and 'low' when lower than the median serum T-cho.

pathological studies

Biopsied samples collected before treatment were fixed in formalin, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin for morphological analysis. All diagnoses were made according to the World Health Organization classification [15]. Cases involving transformed low-grade/indolent B-cell lymphoma were excluded. All the samples were reviewed by expert hematopathologists (KT and TY).

treatment

For patients with stage IB–IV, rituximab was administered at the standard dose of 375 mg/m^2 in 8-week cycles during the first and second cycles of CHOP or six triweekly cycles concurrent with each of the six cycles of triweekly CHOP, as described previously [16]. Patients with stage IA were treated with three cycles of CHOP with subsequent radiotherapy; rituximab was administered in 8-week cycles during the first and second cycles of CHOP or three triweekly cycles concurrent with each of the three cycles of triweekly CHOP.

statistical analysis

Progression-free survival (PFS) was calculated from the date of RCHOP initiation to the date of documented disease progression, relapse, or the end date of the study. Overall survival (OS) was calculated from the date of RCHOP initiation until death from any cause or the last follow-up. If the stop date was not reached, the data were censored at the date of the last follow-up evaluation. Survival curves were created by the Kaplan–Meier method; overall differences were compared by the log-rank test. Cox multivariate analysis was carried out to estimate the prognostic impacts of statin use, cholesterol profile, and IPI on PFS and OS. Comparisons of the basic characteristics of each group (with and without statin use) were made using Fisher's exact test, the chi-square test, and the Mann–Whitney *U* test. The data were analyzed using SPSS software (version 11.0 for Windows; SPSS, Chicago, IL).

results

Of the 256 patients who met the inclusion criteria, CD20 expression was confirmed in all DLBCL patients by immunohistochemical staining and flow cytometry. The characteristics of the patients are listed in Table 1. A total of 35 patients (14%) received statins (atorvastatin in 17 cases, pravastatin in nine cases, simvastatin in five cases, and pivastatin and rosuvastatin in two cases each). In the statin group, all patients started statin therapy before RCHOP initiation, and the median duration from initiating statin therapy to RCHOP therapy was 38 months (range 3–63 months). In the no-statin group, patients did not intake any statins after RCHOP initiation. Most patients took statins once

Table 1. Patient characteristics

Clinical parameter	Statin use		*P*
	Yes, no. (%)	No, no. (%)	
All	35	221	
Sex			0.02
Male	13 (37)	130 (59)	
Female	22 (63)	91 (41)	
Age, years			0.08
Median (range)	68 (20–86)	64 (23–88)	
≤60	6 (17)	71 (32)	
>60	29 (83)	150 (68)	
Stage			0.46
1–2	18 (51)	131 (59)	
3–4	17 (49)	90 (41)	
PS			1.00
0–1	33 (94)	204 (92)	
2–4	2 (6)	17 (8)	
LDH			0.36
Normal	16 (46)	121 (55)	
High	19 (54)	100 (45)	
No. of extranodal sites			1.00
0–1	28 (80)	172 (78)	
2–	7 (20)	49 (22)	
IPI score			0.44
0–2	22 (63)	153 (69)	
3–5	13 (37)	68 (31)	
Time from diagnosis to treatment, days (range)	19 (2–34)	18 (0–94)	1.00
T-cho			
Median (range)	210 (141–318)	187 (101–296)	0.16
TG			
Median (range)	156 (48–534)	112 (40–790)	0.37
HDL-C			
Median (range)	49 (25–92)	48 (13–124)	0.86

PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase, U/l; IPI, International Prognostic Index; T-cho, total cholesterol, mg/dl (normal range 135–219); TG, triglyceride mg/dl (normal range 0–150); HDL-C, high-density lipoprotein cholesterol, mg/dl (normal range 40–80).

daily every day. The serum T-cho level tended to be higher for those patients taking statins than for those patients not using statins; however, the difference was not statistically significant. No significant difference was found between the two groups in terms of their basic characteristics except for sex: females were the dominant statin users.

The complete remission rate was not significantly different between the patients with (74%) and without (73%) statin use. With a median follow-up of 32 months (range 7–57 months), the Kaplan–Meier method revealed that the 3-year PFS rates were 84% and 73%, while the OS rates were 89% and 78% for the groups with and without statin use, respectively. Although both survival rates were superior in the statin group, the differences were not statistically significant ($P = 0.38$ and 0.28, respectively; Figure 1A and B). Similarly, the PFS and OS rates at 3 years were 75% and 87%, respectively, for the low-cholesterol group and 85% and 90%, respectively, for the high-cholesterol group; no significant differences in these parameters were observed between the two groups ($P = 0.39$ and $P = 0.28$, respectively). On the other hand, the 3-year PFS rates were 60% and 81% ($P = 0.005$), while the OS rates were 69% and 85% ($P = 0.004$) for the high- and low-IPI groups, respectively, and this result was not affected after adjusting for statin use (data not shown). There were no significant differences in PFS and OS between longer and shorter statin users who had statins for more and less than median duration, respectively ($P = 0.58$ and $P = 0.94$, respectively). To adjust for the prognostic value of serum cholesterol level and IPI, Cox multivariate analysis was carried out. As shown in Table 2, statin use did not have a significant prognostic value either for PFS or OS after adjusting for serum cholesterol level. Of 26 deaths, 21 were caused by progressive disease, while two were caused by interstitial pneumonia, two by bacterial infection, and one by suicide; no patient died of a cardiovascular event.

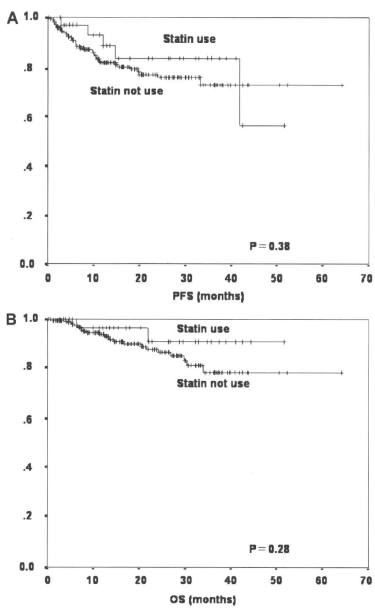

Figure 1. Progression-free survival (PFS) and overall survival (OS) curves for diffuse large B-cell lymphoma patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone according to statin use. PFS (A) and OS (B) curves after statin use ($n = 35$) compared with no statin use ($n = 221$).

discussion

Our data indicate no significant influence of statin use on prognosis in patients with DLBCL. Indeed, there is currently no report confirming the clinical significance of statins in patients treated with rituximab.

Our results do not support the previous laboratory data, and several explanations can be offered for these discrepancies. First, the cholesterol levels of the patients may not have been adequately lowered by the statin doses given. However, in this study, the general outcome was not affected even in the low-cholesterol group. Thus, it is important to determine the *in vivo* threshold level of cholesterol, if any, that affects the CD20 conformation reportedly required for rituximab-mediated CDC and ADCC [9]. Second, accumulating evidence indicates that statins have anticancer [17, 18] and antilymphoma activity [19, 20]. Therefore, statin use may have favorably influenced the RCHOP-treated DLBCL patients in this study. Third, several prognostic markers have been assessed and identified in patients with DLBCL treated with rituximab-combined chemotherapy [21–24]. Consistent with recent findings [21, 22, 25], the IPI score also had a prognostic impact on outcome in our cohort of patients who received immunochemotherapy; thus, biochemical markers and clinical prognostic factors may have greater predictive power than statin use.

Similar to statin use and serum cholesterol in this study, no prognostic marker other than IPI has been found to predict

Table 2. Cox multivariate analysis for PFS and OS

Variable	HR	95% CI	*P*
PFS			
Statin use	0.85	0.31–1.51	0.35
Cholesterol level	0.01	0.01–0.12	0.98
IPI	2.69	0.88–4.08	0.10
OS			
Statin use	0.52	00.29–1.90	0.52
Cholesterol level	0.001	0.00–0.00	0.98
IPI	6.55	1.34–8.89	0.01

HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; IPI, International Prognostic Index; OS, overall survival.

prognosis after rituximab was introduced into clinical practice. Nevertheless, a number of prognostic markers have been identified in patients with DLBCL treated with chemotherapy alone [26–29], some of which have been reassessed and shown to be unassociated with prognosis in patients treated with rituximab-combined chemotherapy [21–23]. BCL2 overexpression was reportedly associated with poorer survival in patients treated with CHOP-like regimens [26, 27], but several studies failed to confirm its prognostic value in patients treated with rituximab-combined chemotherapy, indicating that the addition of rituximab overcomes the negative influence of BCL2 overexpression [23]. BCL6, a marker of germinal center derivation, has been identified as a marker of a favorable outcome in DLBCL [28], but outcomes for patients treated with immunochemotherapy were not influenced by BCL6 status [21]. Similarly, no correlation was observed between the immunohistochemically defined GC phenotype and survival rate in patients receiving immunochemotherapy [29], which is in contrast to previous findings of inferior outcomes in non-GC patients relative to GC patients in the pre-rituximab era [22].

Although our study was not a randomized, prospective study and was possibly biased by factors other than statin use, the distribution of baseline characteristics, including IPI factors, was similar between the groups. Unfortunately, the sample size of statin users was likely too small to reach any definitive conclusions. In this study HRs of statin use for PFS and OS were 0.85 and 0.52 without statistical significance. Post hoc statistical power calculation revealed that the statistical power for these HRs were 5.9% and 35.6%, respectively. Therefore, one may say that we need more studies to draw more solid conclusion. Furthermore, our analysis is based solely upon patients with DLBCL receiving RCHOP therapy and may not be generalizable to patients with other types of B-cell lymphoma or patients receiving rituximab alone.

In conclusion, we investigated the effects of statin use on clinical outcome in DLBCL patients receiving RCHOP and found that statin use did not influence their prognosis. To confirm this conclusion, larger scale, prospective studies of DLBCL patients are required.

acknowledgements

The authors thank Yuko Mishima, Sakura Sakajiri, Takashi Saotome, and Shunji Takahashi from the Cancer Institute Hospital and Shiro Kubonishi, Eisei Kondo, and Masami Niiya from Okayama University for treating the patients and Daigo Shoji, Chie Watanabe, Tizuru Suitu, Ayako Sakai, and Michiko Ennishi for collecting the clinical data.

Author contributions: DE designed the study, treated the patients, collected clinical data, and wrote the paper; HA designed the study, treated the patients, and collected clinical data; KT and YT scored the immunohistochemical staining, designed the study, and wrote the paper; MY treated the patients, assisted in designing the study, and writing the paper; YM, KS, and YT treated the patients and supervised the paper; KM made the statistical analysis and wrote the paper; and KI, KH, and M.T. designed the study, supervised all aspects of the research and analyses, and wrote the paper.

disclosure

The authors declare no conflict of interests and funding sources.

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Hematological Oncology
Hematol Oncol 2010; **28**: 27–32
Published online 8 April 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/hon.896

Research Article

The therapeutic effect of rituximab on CD5-positive and CD5-negative diffuse large B-cell lymphoma

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Received: 14 December 2008
Revised: 3 January 2009
Accepted: 13 January 2009

Abstract

The prognosis of diffuse large B-cell lymphoma (DLBCL) has improved markedly in recent years of rituximab era. The prognosis of *de novo* CD5-positive DLBCL is reported to be poor, but the effect of rituximab on this type of lymphoma remains unclear. To investigate the effect of rituximab on CD5-positive DLBCL, we collected DLBCL patients and analysed prognostic factors. A total of 157 patients with DLBCL who were immunophenotyped with flow-cytometry (FCM) and treated with chemotherapy were subjected to analysis. Those treated with radiotherapy alone or with supportive therapy only were not included. Patients diagnosed in 2003 or later were treated with rituximab combined chemotherapy. There were 95 males and 62 females. Their age ranged from 20 to 91 years old, and the median was 65 years. Nineteen patients were diagnosed as having *de novo* CD5-positive DLBCL. Rituximab was given alongside chemotherapy in 85 patients. Of these, 11 were positive for CD5 and 74 were negative. The addition of rituximab improved the overall survival (OS) of DLBCL patients (2-year OS: 82% vs. 70%, $p = 0.01$). For CD5-negative DLBCL, patients treated with rituximab showed 2-year OS of 84%, which was significantly better than those treated without rituximab (70%, $p = 0.008$). However, for CD5-positive DLBCL, the prognosis was not statistically different between the patients treated with and without rituximab (59% vs. 50%, $p = 0.72$). Although rituximab improved the prognosis of DLBCL, such improvement was restricted to the CD5-negative group. Further investigation is required to improve the prognosis of patients with CD5-positive DLBCL. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: **rituximab; CD5; B-cell; lymphoma; BCL-2; prognosis**

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the largest category of malignant lymphoma and is now considered to be made up of several heterogeneous subgroups. [1,2] *De novo* CD5-positive DLBCL is a distinct subtype of DLBCL that is characterized by elderly onset, female predominance, an advanced stage of disease at diagnosis, the presence of B symptoms, a high lactate dehydrogenase (LDH) level, frequent extranodal involvement and poor prognosis. [3–8] Immunoglobulin mutation analysis of *de novo* CD5-positive DLBCL has identified that this lymphoma is derived from the post germinal centre (GC) stage of somatically mutated B-cells. [3,4,9] Genetic analyses using comparative genomic hybridization (CGH) and microarray technologies have further shown that *de novo* CD5-positive DLBCL is distinct from CD5-negative DLBCL and mantle cell lymphoma (MCL). [10–12]

Patients with advanced stage DLBCL used to be treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) based chemotherapy. [13,14] The prognosis of DLBCL has improved markedly in recent years of rituximab era. [15–19] However, the effect of rituximab-containing chemotherapy on *de novo* CD5-positive DLBCL remains unclear. To investigate the effect of rituximab on this type of lymphoma, we collected patients that were suffering from DLBCL and analysed prognostic factors.

Methods

Patient selection

From April 1998 to October 2006, a total of 424 patients were newly diagnosed with *de novo* DLBCL in the Yokohama City University School of Medicine and

collaborating institutes. Of these, 157 were analysed for surface markers with flow-cytometry (FCM) and received chemotherapy. Patients diagnosed before 2003 were previously reported. [20] Those treated with radiotherapy alone or with supportive therapy only were excluded from this study. Patient therapeutic records were retrospectively analysed. The study was approved by the Institutional Review Board from Yokohama City University Medical Center, and complied with the Helsinki Declaration.

Morphological and immunophenotypical analyses

CD5-positive DLBCL was diagnosed when the tumour cells were positive for CD5 according to FCM. All of the CD5-positive DLBCL specimens were re-examined histopathologically. Tissue was fixed in 10% formalin and embedded in paraffin. The sections (4 μm thick) were stained with hematoxylin and eosin, and immunostained for CD5 (4C7, Novocastra, Newcastle-upon-Tyne, UK), CD10 (56C6, Novocastra), CD20 (L26, Dako, Glostup, Denmark), BCL2, (124, Dako), BCL6 (PG-B6p, Dako), MUM1 (MUM1p, Dako) and cyclinD1 (P2D11F11, Novocastra) using the dextran-polymer method (EnVision+; Dako, Glostup, Denmark). For immunohistochemistry, heat-induced antigen retrieval pretreatment using Target Retrieval Solution, pH 9 (Dako) was carried out. Histologic specimens from all of the patients with CD5-positive DLBCL were reviewed by hematopathologists, including one of the authors (K.T.), according to the WHO classification. [2] All patients were diagnosed as having DLBCL. Patients diagnosed with intravascular lymphoma (IVL) were not included for this study. One patient diagnosed with DLBCL with an intravascular pattern was included because the area of DLBCL was the most prominent.

In immunohistochemical evaluation, BCL6, MUM1 and BCL2 were defined as positive when the proportion of stained lymphoma cells was 30% or higher. CD5 and CD10 were considered to be immunohistochemically positive when at least a small population of the neoplastic cells was positive. Subtype of GC or non-GC phenotype was judged using CD10, BCL6 and MUM1 according to the Hans's criteria. [21]

Treatment of patients

All patients diagnosed before 2003 were treated with chemotherapy with standard CHOP or CHOP-like anthracycline-containing regimens. In 2003 or later, patients were treated with rituximab containing chemotherapy (CHOP-R or others with rituximab). Fourteen patients were treated with additional radiotherapy (10 patients without rituximab and four patients with rituximab) and nine patients received autologous transplantation (seven patients without rituximab and two patients with rituximab). All but one patient who received additional therapy was diagnosed as having CD5-negative DLBCL.

Statistical analysis

Correlations between the two groups were examined with the χ^2 test, the Fisher exact test, and the Mann–Whitney *U*-test. Patient survival data were analysed with the Kaplan-Meier method and were compared by means of the log-rank test. Overall survival (OS) was defined as the time from diagnosis to the date of death or last contact. Progression free survival (PFS) was defined as the time from diagnosis to the date of progression, relapse, death or last contact. Univariate and multivariate analyses were performed with the Cox proportional hazard regression model. Variables were selected with the step-wise method. Data were analysed with Fisher (Nakayama-Shoten, Tokyo, Japan) and STATA statistical software (College Station, TX).

RH contributed to the design of the study, collected clinical data, analysed the data and wrote the manuscript. NT designed the study and collected clinical data. KT designed the study and wrote the manuscript. TA, AF, HK, CH, ST, JT, RS, HF, SF, KO and SM collected clinical data. RS wrote the manuscript. YI supervised the study and gave critical advice.

Results

Patient characteristics

There were 95 males and 62 females. Their ages ranged from 20 to 91 years old, and the median age was 65 years. The median follow-up of the surviving patients was 2.2 years. Nineteen patients were diagnosed as having *de novo* CD5-positive DLBCL (Table 1). Of these, the expression of CD5 by FCM was bright in 16 patients and dim in three. In comparison with CD5-negative DLBCL, the CD5-positive group had high serum lactate dehydrogenase (LDH) (95%, $p = 0.02$) and soluble IL-2 receptor levels (63%, $p = 0.0001$), advanced stage (84%, $p = 0.01$), a high International Prognostic Index (IPI) score (79%, $p = 0.01$) and also displayed B-symptoms (68%, $p = 0.002$). Rituximab was added to the chemotherapy in 85 patients. Of these, 11 were positive for CD5 and 74 were negative (Table 1).

In the patients treated with rituximab, there were 55 males and 30 females. Eleven patients were diagnosed as having *de novo* CD5-positive DLBCL (M/F = 5:6). In comparison with the CD5-negative DLBCL group, the CD5-positive group had a high soluble IL-2 receptor level (73%, $p = 0.001$), advanced stage disease (91%, $p = 0.01$), a high IPI score (91%, $p = 0.006$) and also displayed B symptoms (73%, $p = 0.006$).

Immunohistologic features

Among the 19 CD5-positive DLBCL patients, CD5 was positive in 14 patients (74%) according to immunohistochemistry. The expression was weak or focal in nine of the 14 patients. All of the 19 CD5-positive patients were negative for Cyclin D1 and positive for CD20. Only two of the patients were positive for CD10. Another two patients that were negative for CD10 were positive for BCL6 and

Table 1. Patient characteristics of CD5-positive and CD5-negative DLBCLS

Characteristics	CD5 + DLBCL ($n = 19$)	CD5 − DLBCL ($n = 138$)	p-value
Sex (male/female)	8/11	87/51	0.08
Age: median	66	64	
Range	30–91	20–88	
>60 years	14 (74)	83 (60)	0.25
Performance status >1	9 (47)	42 (30)	0.13
LDH: elevated	18 (95)	99 (72)	0.02
Advanced stage	16 (84)	76 (55)	0.01
No. of extranodal involvement >1	10 (53)	46 (33)	0.09
IPI: high-intermediate/high	15 (79)	69 (50)	0.01
B symptoms: present	13 (68)	45 (33)	0.002
Bulky mass: present	2 (11)	42 (30)	0.05
Soluble IL-2 receptor >5000	12 (63)	32 (23)	0.0001
Rituximab (−/+)	8/11	64/74	0.91

Values in parentheses indicate the percentage of patients.
DLBCL, diffuse large B-cell lymphoma; LDH, lactate dehydrogenase; IPI, International Prognostic index; IL-2, interleukin-2.

negative for MUM1. Therefore, a total of four patients with CD5-positive DLBCL showed the GC phenotype (21%). All but one of the CD5-positive patients were positive for BCL-2 (95%). Ki67-positive cells ranged from 50 to 70% except in one patient who had an extremely low positivity of 10%.

Therapeutic response and prognosis

The patients who were treated with rituximab showed significantly better OS that those treated without rituximab. Two-year OS was 82% for the former and 70% for the latter ($p = 0.01$, Figure 1A), and the respective 2-year PFSs were 66 and 53% ($p = 0.04$, Figure 1B). For CD5-negative DLBCL, the addition of rituximab significantly improved patient prognosis. Patients treated with rituximab showed 2-year OS of 84%, which was significantly better than that for patients treated without rituximab (70%, $p = 0.008$, Figure 2A). However, for CD5-positive DLBCL, the prognosis was not statistically different between the patients treated with and without rituximab (59% vs. 50%, $p = 0.70$, Figure 2B). Although rituximab improved the prognosis of DLBCL, such improvement was restricted to the CD5-negative group.

Univariate Cox analysis identified the following prognostic factors for all patients: performance status (PS), serum LDH level, clinical stage, extranodal involvement at

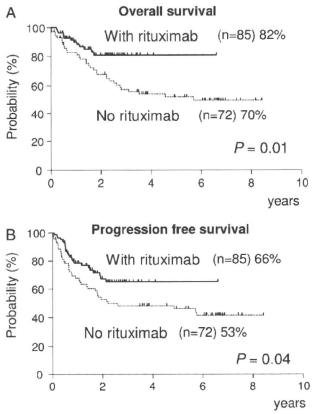

Figure 1. Survival curves of DLBCL patients treated with or without rituximab. The patients treated who received rituximab showed significantly better overall survival (A) and progression free survival (B) than those treated without rituximab

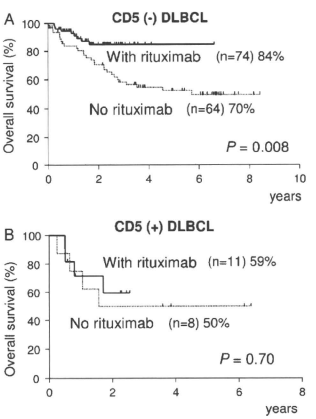

Figure 2. Overall survival curves of CD5-positive and -negative DLBCL patients treated with or without rituximab. (A) The prognosis of CD5-negative DLBCL patients was significantly improved by the use of rituximab. (B) In contrast, no beneficial change was observed for CD5-positive DLBCL patients after the introduction of rituximab

Table 2. Prognostic factors affecting overall survival

Variables	Unfavourable factors	Univariate			Multivariate		
		HR	95%CI	*p*-value	HR	95%CI	*p*-value
CD5 status	Positive	—	NS				
Age	>60 years	—	NS				
PS	>1	3.7	(2.1–6.7)	<0.0001	2.3	(1.2–4.2)	0.007
LDH	> normal	9.8	(2.3–40.8)	0.001	8.7	(2.0–36.9)	0.003
Stage	III/IV	2.4	(1.2–4.8)	0.01	—	NS	
Extranodal sites	>1	2.3	(1.3–4.2)	0.004	—	NS	
B symptoms	Present	2.7	(1.4–4.9)	0.001	2.2	(1.1–4.1)	0.01
Bulky mass	Present	2.1	(1.1–3.9)	0.01	—	NS	
Rituximab	Not used	2.4	(1.2–5.0)	0.01	2.5	(1.3–5.0)	0.006

PS, performance status; LDH, lactate dehydrogenase; HR, hazard ratio; CI, confidence interval.

more than one site, the presence of B symptoms, the presence of a bulky mass and the induction of rituximab (Table 2). Multivariate analysis showed that PS, serum LDH level, the presence of B symptoms and the induction of rituximab were independent prognostic factors (Table 2). For patients treated with rituximab, univariate analysis identified that PS (hazard ratio (HR): 6.1, 95% confidence interval (CI): 1.8–20.0, $p = 0.002$) and the presence of B symptoms (HR: 3.5, 95%CI: 1.1–10.9, $p = 0.02$) were prognostic factors. Multivariate analysis showed PS was the only independent prognostic factor (HR: 5.0, 95%CI: 1.2–21.1, $p = 0.02$). CD5 status was not identified as significantly prognostic by univariate or multivariate analysis either in all or rituximab-treated patients.

Discussion

The addition of rituximab to anthracycline-containing chemotherapy has improved the prognosis of B-cell lymphomas including DLBCL. [15–19] Our current study shows that this effect of rituximab is only seen in CD5-negative DLBCL patients.

Several factors are known to be prognostic for DLBCL, [2,22,23] and the most notable clinical factor is the IPI score. [24] In recent rituximab era, however, the prognostic model of IPI has been reclassified into a novel three prognostic groups, which is termed the revised-IPI (R-IPI). [25] In addition, the concept of a 'biological prognostic factor' has emerged, [26] and gene expression profiling using microarray technologies has significantly altered the assessment of biomarkers. [27–29] Gene expression profiling using microarray technology has resulted in DLBCL being divided into two major subgroups: one similar to normal germinal centre B cells (GCB) and the other similar to activated peripheral blood B cells (ABC). Hans *et al.* [21] proposed a model using the presence of three proteins (CD10, BCL6 and MUM1) to discriminate between GCB-type and ABC-type DLBCL. Lossos *et al.* [30] proposed another prognostic model using the expression of six predictive genes (*LMO2, BCL6, FN1, CCND2, SCYA3* and *BCL2*). Other investigators have also claimed that BCL2 expression predicts a poor prognosis. [31–33] Recently, the introduction of rituximab has been reported to improve the poor prognosis of BCL2-positive DLBCL. [34–37] Therefore, rituximab is believed to have the potential to affect prognostic factors.

CD5-positive DLBCL accounts for 5–10% of all DLBCLs and is associated with female predominance, an advanced stage of disease at diagnosis, and an aggressive disease course, and it is reported that these characteristics cause poor prognosis. [8,38] However, CD5 status was not shown to be an independent prognostic factor by multivariate analysis. [8] Because CD5 expression is closely correlated with each of the factors that make up the IPI score, CD5 expression in DLBCL represents a biological feature of aggressiveness, which is also detectable by means of these clinical parameters. The present results are consistent with the previous findings in the literature. Notably, these features are concordant with GCB/ABC categorization since most CD5-positive DLBCLs are included in ABC-type DLBCLs.[39] Our study showed CD5-positive DLBCL frequently expresses BCL2, which is consistent with the literature findings that the expression of BCL2 is significantly more frequent in CD5-positive DLBCL than in CD5-negative DLBCL. [40,41] Because the induction of rituximab improves the poor prognosis of BCL2-positive DLBCL, [34–37] we initially postulated that the improvement in prognosis would be more prominent in CD5-positive DLBCL. However, the prognosis of CD5-positive patients did not improve after supplementation with rituximab. These findings suggest that the favourable outcome of rituximab containing chemotherapy is not mediated by BCL2. Our findings as well as those of Ennishi, *et al.* [41] showed the prognosis of CD5-positive patients was poor with rituximab treatment. Further studies are required to clarify the reasons for the poor prognosis of CD5-positive DLBCL patients.

In the literature, CD5-positive DLBCL has been described to have a close correlation with intravascular large B-cell lymphoma (IVL). [8,40] Approximately 30–40% of CD5-positive DLBCL shows a focal intravascular or intrasinusoidal pattern of lymphoma infiltration. On the other hand, varying degrees (22–75%) of CD5 expression have been reported for IVL. [42–46] In the current study, we excluded patients who were diagnosed with IVL so we could strictly focus on CD5-positive DLBCL. Recently, the addition of rituximab to anthracycline containing chemotherapy has been reported to improve the prognosis of

IVL.[47] Our current results for CD5-positive DLBCL are different from those of IVL, suggesting a need for careful differentiation of IVL from *de novo* CD5-positive DLBCL.

Investigation of appropriate treatment strategies for CD5-positive DLBCL is urgently required because the prognostic differences between CD5-positive and -negative DLBCL are expanding. One of the candidates for this is a dose-adjusted EPOCH and rituximab regimen that is more effective in patients with poor prognostic DLBCL. [48,49] Another candidate is a combination of rituximab and ACVBP chemotherapy that is reported to be effective for central nervous prophylaxis. [50] Prospective studies of these novel regimens are thus warranted.

In conclusion, rituximab improves the prognosis of CD5-negative DLBCL patients when combined with standard chemotherapy, but does not do the same for CD5-positive DLBCL patients.

Acknowledgements

The authors thank Juichi Tanabe for his aid in collecting clinical data from the Fujieda Municipal General Hospital. KT is a consultant of Dako. Other authors declare that they have no potential conflicts of interest.

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Hematol Oncol 2010; **28**: 27–32
DOI: 10.1002/hon

Breast Cancer
DOI 10.1007/s12282-010-0213-x

CASE REPORT

Hepatitis B virus reactivation in adjuvant chemotherapy for breast cancer

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Received: 29 October 2009 / Accepted: 7 June 2010

Abstract Immunosuppressive therapy, such as chemotherapy or the use of corticosteroids, may stimulate reactivation of hepatitis B virus (HBV). Most of these episodes occur in patients whose hepatitis B surface antigens are positive (HBsAg+). We report a case of HBV reactivation in a patient with negative HBsAg during chemotherapy for breast cancer, in spite of avoiding corticosteroids. A 68-year-old woman received adjuvant chemotherapy for breast cancer. Her serological examinations showed that HBsAg, HBeAg, and HBV-DNA were all negative. Her chemotherapy consisted of CAF (cyclophosphamide 500 mg/m^2, doxorubicin 50 mg/m^2, fluorouracil 500 mg/m^2) without administration of corticosteroids. She received six cycles of CAF. At day 27 after her sixth CAF, she was admitted to the hospital with acute hepatitis B virus (HBV) reactivation. She received glycyrrhizinic acid by intravenous injection (80 mg/day), ursodeoxycholic acid (300 mg/day), and entecavir (0.5 mg/day). Then she received interferon by intravenous injection (3 million units/day), prednisolon by intravenous injection (45 mg/day), and plasma exchange therapy. However, she died of liver failure. This is a rare case in which HBV reactivation occurred in an HBsAg negative patient during chemotherapy without using corticosteroids. This episode suggests that HBV reactivation may occur during chemotherapy in any patient with a history of HBV infection. Therefore, we recommend checking HBsAg, HBsAb, and HBcAb before starting chemotherapy. Moreover, with positive HBsAb or/and HBcAb patients, HBV-DNA should be checked before starting chemotherapy and monitored during chemotherapy by a sensitive PCR method.

Keywords HBV reactivation · Breast cancer · Chemotherapy

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Introduction

Recently many patients with breast cancer have received anthracycline-containing chemotherapy as adjuvant chemotherapy and as a treatment for metastatic cancer. Because chemotherapy is a toxic therapy, it is important not only to judge which patients could receive benefit from chemotherapy but also to judge whether the patient could receive the therapy safely.

It is well known that HBV reactivation may occur in the patients receiving chemotherapy for malignant lymphoma, and it is effective to use antiviral drugs prophylactically to prevent HBV reactivation. It should be known that HBV reactivation may often occur during chemotherapy in HBsAg negative patients who have been infected by HBV

Published online: 24 July 2010

in their past. For the HBsAg negative and HBV-Ab negative patients, it is less common and less known but it could occur [1–3].

Several articles have been published about HBV reactivation during breast cancer chemotherapy and a few have reported that prophylactic antiviral drugs were effective for prevention of HBV reactivation in HBsAg positive patients [4–6]. But, it has never been reported that HBV reactivation may occur in a patient with negative HBsAg during breast cancer chemotherapy. And, methods for screening for HBV before starting chemotherapy and how to follow during or after chemotherapy have not yet been established.

Case report

A 68-year-old woman with breast cancer received a partial mastectomy and biopsy of the sentinel nodes in our hospital. The resected breast tumor revealed a scirrhous carcinoma, 2.1 cm, with slight lymphatic permeation and moderate fat infiltration. The resected node was free from cancer. Estrogen and progesterone receptors were both positive. HER2 was negative, with a HercepTest score of 2+, but no amplification of HER2 gene by fluorescence in situ hybridization (FISH) method was observed. The risk of recurrence, when endocrine therapy alone was applied, was estimated to be approximately 20% in 15 years [7]. Thus, we planned chemotherapy followed by endocrine therapy. She was treated with six cycles of CAF. The CAF regimen consists of cyclophosphamide 500 mg/m^2 on day 1, doxorubicin 50 mg/m^2 on day 1, and fluorouracil 500 mg/m^2 on days 1 and 8. This was repeated six times every 3 weeks.

No dexamethasone as antiemetic was given, because she was a carrier of HBV, although her HBsAg was negative, showing no evidence of cirrhosis. We screened for HBV-DNA, but the score was below the sensitivity level of the PCR method (Fig. 1). In the fifth cycle of CAF, she complained of slight fatigue with normal levels of aminotransferase. At the sixth cycle of CAF, her aminotransferase level increased over standard for the first time; aspartate aminotransferase (AST) was 44 U/L, alanine aminotransferase (ALT) 29 U/L, and total bilirubin 0.4 mg/dL (Fig. 1). Chemotherapy was given, but she showed vertigo and could not receive the day 8 fluorouracil injection.

Eleven days after the last CAF, she got a fever and received an antibacterial drug and granulocyte colony-stimulating factor. Two days afterwards, she complained of loss of appetite. White blood cell count (WBC) was 1200/μL, C-reactive protein (CRP) 1.7 mg/dL, AST 87 U/L, ALT 66 U/L. There was no additional laboratory information of diagnosed hepatitis. She received conservative treatment for 5 days without antiviral drugs. Her general condition and appetite improved, and she was discharged from our hospital.

Six days later, AST, ALT and bilirubin were 358 U/L, 127 U/L, 2.0 mg/dL, respectively. We considered that her elevated transaminoferase was due to drug-induced hepatic dysfunction. Four days after that, AST was 321 U/L,

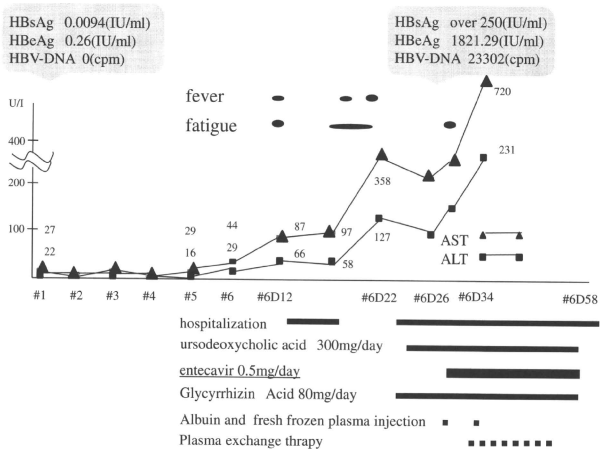

Fig. 1 Summary of the patient's laboratory data and symptom during chemotherapy, and our treatment during two hospitalizations. HBsAg, HBeAg, and HBV-DNA were all negative before starting chemotherapy, but turned positive at the sixth cycle of CAF. Aminotransferase level increased progressively. She died of liver failure in spite of intensive care

ALT 120 U/L, and bilirubin 4.1 mg/dL. We performed additional laboratory examination, and her HBsAg was over 250 IU/mL, and HBeAg 1821 IU/mL (Fig. 1). She was admitted to our hospital again, receiving glycyrrhizinic acid intravenous injection (80 mg/day), ursodeoxycholic acid (300 mg/day) to protect the liver, and entecavir (0.5 mg/day) (Fig. 1). But her liver function slowly worsened. We transferred her to a hospital specializing in treating liver failure. She received intensive care, entecavir (0.5 mg/day), interferon by intravenous injection (3 million units/day), prednisolon by intravenous injection (45 mg/day), and plasma exchange therapy. But she did not recover and died of liver failure.

Discussion

It is well known that immunosuppressive therapy may stimulate reactivation of HBV. Most of these episodes occur in patients whose HBsAg is positive. HBV reactivation occurs in 12–53% of HBsAg+ patients [1–3, 8]. However, there is a small but definite risk in patients who seem to have cleared HBV infection with hepatitis B core antibody positive (HBcAb+), hepatitis B surface antigen negative (HBsAg−) [3, 9]. This phenomenon is thought to result from the persistence of HBV in the liver, despite apparent serological clearance [1–3, 7]. Thus, screening only HBsAg before starting chemotherapy is not enough to evaluate the risk of HBV reactivation. It is recommended to check HBsAg, HBcAb, and HBsAb before chemotherapy for hematological malignancies [1, 9]. It is also recommended that HBcAb or HBsAb positive patients should be monitored during chemotherapy not only for transaminase but also for HBV-DNA, because virus replication occurs before the symptom of hepatitis with elevation of transaminase [3, 10], and it is too late to start treatment with hepatitis after complete replication.

Our patient had suffered from hepatitis B in 1989 and was HBsAg(+), HBsAb(−), HBeAg(−), HBeAb(+), HBcAb(+). We examined HBV-DNA before starting chemotherapy but did not monitor it, nor did we not check HBcAb. In the present case, the result of HBV-DNA might have been a false negative due to insensitivity of the transcription-mediated amplification (TMA) method. The TMA method cannot detect less than 2.6 log copies/mL of HBV-DNA. In contrast, the real-time PCR method is able to detect over 1.8 log copies/mL. It is therefore preferred to use a more sensitive method such as real-time PCR when monitoring HBV-DNA.

The risk of HBV reactivation in solid cancer patients is less clearly established. HBV reactivation in patients with solid tumors occurred in 15% of HBsAg+ patients [3]. Reactivation occurred in 41–56% of the patients with breast cancer with HBsAg+ [3]. To our knowledge, there is no report about reactivation in HBsAg negative patients during chemotherapy for breast cancer. Immunosuppressive therapy has the potential to allow HBV replication. After recovery of the host's immune response, hepatitis could occur as an immune reaction to remove HBV. Once acute hepatitis has occurred, some cases may develop fulminant hepatitis or hepatic failure. One Chinese prospective study reported that HBV reactivation during chemotherapy for malignant lymphoma occurred in 48% of HBsAg+ patients, of whom 23% developed icteric hepatitis, and 7.4% developed hepatic failure, of whom 50% died [3].

Several reports suggested the effect of prophylactic lamivudine on hepatitis B reactivation during chemotherapy [4, 6]. Yeo et al. reported that in the prophylactic lamivudine group, there were significantly fewer incidences of hepatitis (12.9 vs. 59%, $p < 0.001$), less HBV reactivation (6.5 vs. 31.1%, $p = 0.008$), and less disruption of chemotherapy (16.1 vs. 45.9%, $p = 0.006$). Loomba et al. [11] reported that prophylactic therapy with lamivudine may reduce the risk of HBV reactivation and HBV-associated morbidity and mortality.

In hematologic malignancies, corticosteroid and anthracyclines are the most important drugs associated with HBV reactivation [8]. Recently many breast cancer patients have received anthracycline-containing chemotherapy. Corticosteroids are commonly used with anthracyclines as antiemetic drugs, because anthracycline-containing regimens have a high emetic risk. There is no report about reactivation

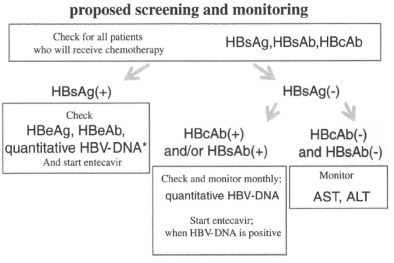

Fig. 2 Proposed screening and monitoring to prevent reactivation of HBV from the guideline [9]. It is recommended to examine not only for HBsAg but also HBcAb and HBsAb before starting chemotherapy for all patients. In either HBcAb or HBsAb positive patients, HBV-DNA should also be checked. HBV-DNA positive patients have to commence entecavir. In HBV-DNA negative patients, we recommend monitoring not only for transaminase but also for HBV-DNA during chemotherapy. When HBV-DNA turns positive, the patient should begin entecavir