別紙４

研究成果の刊行に関する一覧表

雑誌

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Kato H, Morishima Y	Clinical impact and predisposing factors of delayed-onset neutropenia after autologous hematopoietic stem-cell transplantation for B-cell non-Hodgkin lymphoma	Ann Oncol.	—	Feb 19. [Epub ahead of print]	2010
Chihara D, Morishima Y	Primary gastric diffuse large B-cell Lymphoma (DLBCL): analyses of prognostic factors and value of pretreatment FDG-PET scan.	Eur J Haematol.	—	Feb 9. [Epub ahead of print]	2010
Oki Y. Morishima Y	Low absolute lymphocyte count is a poor prognostic marker in patients with diffuse large B-cell lymphoma and suggests patients' survival benefit from rituximab.	Eur J Haematol.	81(6)	448-53.	2008
Oki Y. Ogura M, Morishima Y	Phase II study of a salvage regimen using cyclophosphamide, high-dose cytarabine, dexamethasone, etoposide, and rituximab in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma.	Cancer Sci.	99(1)	179-84.	2008
Suzumiya J, Uike N	International Peripheral T-Cell Lymphoma Project. The International Prognostic Index predicts outcome in aggressive adult T-cell leukemia/lymphoma: analysis of 126 patients from the International Peripheral T-Cell Lymphoma Project.	Ann Oncol.	20(4)	715-21.	2009
Nagai H, Watanabe T, Uike N,	Remission induction therapy containing rituximab markedly improverd the outcome of untreated mature B cell lymphoma	Brit J Haematol.	143	672-80	2008
Tokuda Y, Watanabe T, Chou T, Ogura M, Kasai M,	Phase III study to evaluate the use of high-dose chemotherapy as consolidation of treatment for high-risk postoperative breast cancer:	Cancer Sci.	99	145-51	2008

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Clinical impact and predisposing factors of delayed-onset neutropenia after autologous hematopoietic stem-cell transplantation for B-cell non-Hodgkin lymphoma: association with an incremental risk of infectious events

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Background: Clinical significance of delayed-onset neutropenia (DON) after autologous hematopoietic stem-cell transplantation (ASCT) has not been well described. We conducted a retrospective cohort study to examine risk factors and clinical impact of DON.

Design and methods: Subjects were consecutive 108 patients with B-cell lymphoma receiving ASCT. We defined DON as absolute neutrophil counts $<1.0 \times 10^9$/l at any point from 30 days onward after ASCT without apparent causes of neutropenia. Documented infectious events were reviewed from 1 to 18 months after ASCT.

Results: Fifty-two percent of patients received rituximab. Cumulative incidence of DON was 50% at 1 year. Rituximab usage was identified as an independent risk factor of DON. A total of 117 infectious events were documented, of which 24 events occurred during DON period. Cumulative incidence of total infectious events was 75% and 42% in the groups with and without DON, respectively ($P = 0.001$). Varicella–zoster virus ($P = 0.033$) and upper respiratory infection ($P = 0.016$) were frequent in the patients experiencing DON. In a multivariable analysis, DON remained a significant factor for total infectious events and upper respiratory infection.

Conclusions: Rituximab usage is an independent risk factor of DON. DON correlates with increased occurrence of infectious events. Careful follow-up would be needed after the onset of DON.

Key words: immunotherapy, neutropenia, rituximab, toxicity, viral infection

introduction

Rituximab is a chimeric monoclonal anti-CD20 antibody containing human immunoglobulin G1 kappa constant regions with murine variable regions [1]. It displays significant therapeutic activity for CD20-positive B-cell non-Hodgkin lymphoma (B-NHL) both as a single agent and in combination with cytotoxic chemotherapy [2–5]. Rituximab can also be used before and after autologous hematopoietic stem-cell transplantation (ASCT) showing powerful antitumor effects and pure graft collection, without additive severe hematological toxicity and delaying time to engraftment after ASCT [6–10].

Delayed-onset neutropenia (DON) has been recently reported as a late complication of rituximab usage [8, 11–15]. Reversible rituximab-related DON tends to occur within 6 months after the last infusion of rituximab. Although some study showed that DON occurred more often in patients receiving rituximab in combination with intensive- or high-dose therapy [15], we sometimes had an experience of DON developing after ASCT even in the patients without rituximab exposure. To what extent rituximab contributes to the occurrence of DON is yet elucidated in the situation of rituximab usage for the patients undergoing ASCT.

Impact of DON is relatively subclinical and little clinical importance after standard chemotherapy [11, 15]. Lemieux et al. [14] reported that its occurrence with the immunosuppressive condition such as post-transplantation might predispose some patients to serious but not life-threatening infectious complication; its clinical impact after ASCT is, however, not well known from large series. We conducted a retrospective study to analyze the clinical significance and risk factors of DON in patients with B-NHL receiving induction chemotherapy with or without rituximab followed by ASCT.

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design and methods

study group

This was an institutional review board-approved retrospective cohort study. Medical records of the consecutive B-NHL treated with high-dose therapy followed by ASCT were reviewed in our institute from 20 June 1991 to 31 January 2007, and a total of 111 patients were selected. The patients who received prior rituximab monotherapy or combination chemotherapy were included, and those were excluded if they had undergone prior transplantation. The patients were also excluded if they had engraftment failure after ASCT, had progressive disease or had received other chemotherapy except rituximab monotherapy within 30 days after ASCT.

study definition

Clinical significance was defined in this study as occurrence of documented infectious events after ASCT. Infectious events were reviewed in all patients between 30 days and 1.5 years or last follow-up after ASCT regardless of DON episodes. Nine factors were included in infectious events: Varicella–zoster virus (VZV) infection, herpes simplex virus (HSV) infection, cytomegalovirus (CMV) antigenemia or infection, fever (>38.0°C), upper respiratory infection, pneumonia (excluding interstitial lung diseases), gastroenteritis, urinary tract infections, and other infectious events. Other infectious events represented local bacterial infections.

DON was defined as absolute neutrophil count (ANC) $<1.0 \times 10^9$/l at any point from 30 days after ASCT to last follow-up without apparent causes of neutropenia after neutrophil engraftment. Patients were counted as censored events if they had apparent causes of neutropenia such as disease progression, drug-related toxic effects or secondary hematologic malignancies, and those were also counted as censored events if they had further chemotherapy after ASCT. Patients were not counted as censored events who had neutropenia followed by infectious events or had the ambiguous onset or who were treated after ASCT with rituximab monotherapy or local radiation therapy for residual diseases. Severity of DON, hematologic toxic effects, and infectious events were evaluated according to the National Cancer Institute—Common Terminology Criteria for Adverse Events version 2. Engraftment was defined as the first of two consecutive days on which the patient's ANC was $\geq 0.5 \times 10^9$/l and unsupported platelet counts were $\geq 20 \times 10^9$/l within 30 days after ASCT.

statistical considerations

Incidence of DON was analyzed by cumulative incidence method. Risk factors of DON were evaluated for 16 factors including rituximab usage, age (≤60 or >60 years), sex, histology (aggressive or indolent), number of extra nodal sites (≤1 or ≥2), performance status (≤1 or ≥2), existence of B symptoms (presence or absence of night sweat, >10% weight loss over 6 months, or recurrent fever >38.3°C), the level of lactate dehydrogenase (normal or elevated), existence of bone marrow involvement, existence of bulky lesion (present or absent, largest diameter of the disease ≥10 cm), prior local radiation therapy, number of prior chemotherapy regimens (≤2 or ≥3), pretransplantation setting (up front or relapse/refractory disease), status at ASCT (complete remission or partial remission), CD34-selected ASCT, and types of conditioning regimens [total body irradiation (TBI)-containing regimens or non-TBI regimens]. Bias due to indication of rituximab treatment was considered by application of propensity score [16]. Risk factors for DON occurrence were explored by Cox proportional hazards model applying stepwise method (P value for removal = 0.10 and that for inclusion = 0.05). Finally, we applied bootstrap resampling method for validation of our analyses (repeating 10 000 times with 108 sampling from original cohort). All the analyses were stratified by quartile of propensity score.

Cumulative incidence of infectious events was estimated by Kaplan–Meier method in this study on the grounds that substituting Kaplan–Meier method evaluating as time-varying covariate for cumulative incidence method produced the same results. Potential risk factors associated with infectious events were analyzed, evaluating as time-varying covariate, for 17 factors including DON in addition to the aforementioned 16 factors. Univariable analyses for infectious events were carried out using the cut-off value of 0.15, and factors associated with significance in univariable analyses were subjected to multivariable analyses (the cut-off value of 0.05). These analyses were carried out with the use of STATA software (version 10; StataCorp LP, College Station, TX).

results

study group

Two patients were excluded because of early disease progression. A patient given rituximab 2.3 years before ASCT was also excluded. One patient who received high-dose therapy but not stem-cell transplantation was included, and all the other patients were eligible. Finally, we proved analyses for a total of 108 patients. Patient characteristics were shown in Table 1. Forty-nine patients had diffuse large B-cell lymphoma, 35 follicular lymphoma, 20 mantle cell lymphoma and 4 other B-NHL. Fifteen (14%) patients had prior rituximab exposure before pretransplantation induction chemotherapy. Seventeen patients who received CD34-selected stem-cell transplantation were all rituximab naive. Two patients had a previous history of fludarabine or cladribine administration, and no patient had a history of alemtuzumab injection. The median number of prior treatment regimens was 2. Fifty-six (52%) patients received rituximab in combination with pretransplantation induction chemotherapy. The patients who had rituximab usage received the last rituximab infusion within 3 months before transplantation (median 45 days, range 28–76). No patients had rituximab given during conditioning therapy, and two patients received rituximab monotherapy after ASCT.

Granulocyte colony-stimulating factor (G-CSF) was administered in all patients until neutrophil engraftment after ASCT, and the median time to engraftment of ANC ($\geq 0.5 \times 10^9$/l) was 10 days (range 7–17 days).

incidence and clinical impact of DON

Cumulative incidence of DON was 50% [95% confidence interval (CI) 40% to 59%] in all patients at 1 year, 66% (95% CI 52% to 77%) with rituximab usage group, and 33% (95% CI 21% to 45%) without rituximab group. DON occurred more often in the group with rituximab usage ($P = 0.003$, log-rank test) (Figure 1). The two patients who had a previous history of fludarabine or cladribine administration experienced DON, one of which was a rituximab-naive patient. The median days of first onset of DON was 71 (range 31–399) after ASCT. The median number of DON occurrence was 2 (range 1–10). The median interval of first onset days of DON to second was 36 days (range 4–320 days). The median value of nadir neutrophil count during DON was 0.446×10^9/l (range $0.009–0.968 \times 10^9$/l) at a median of 96 days (range 32–686 days) after ASCT, and grade 4 neutropenia (neutrophil counts $<0.5 \times 10^9$/l) was observed in 53% of patients (Figure 2a). Fifteen (27%) of 55 DON patients experienced bicytopenia (neutropenia and grade 3 or 4 thrombocytopenia or anemia), 6 of which had pancytopenia (neutropenia, grade 3 or 4 thrombocytopenia and

Table 1. Characteristics of patients with the difference of rituximab usage

Characteristics	Number of patients			*P* value[b]
	All ($N = 108$)	Rituximab usage[a] ($n = 56$)	Without rituximab usage ($n = 52$)	
Sex				0.37
Male/female	68/40	33/23	35/17	
Median age, years (range)	50 (16–67)	52 (19–67)	49 (16–63)	0.26
Age ≥61 years	18	14	4	0.02
Histology				0.39
Diffuse large B-cell lymphoma	49	21	28	
Follicular lymphoma	35	19	16	
Mantle cell lymphoma	20	16	4	
Burkitt lymphoma	2	0	2	
Marginal zone lymphoma	1	0	1	
Lymphoplasmacytoid lymphoma	1	0	1	
Ann Arbor stage				0.33
1/2	15	6	9	
3/4	93	50	43	
B symptoms				0.30
Yes/no/unknown	29/78/1	12/44/0	17/34/1	
Bulky				0.14
Yes/no/unknown	34/72/2	15/41/0	19/31/2	
Lactate dehydrogenase				0.28
High/normal/unknown	54/50/4	27/28/1	27/22/3	
Performance status				0.05
≤1	89	50	39	
≥2	19	6	13	
Extranodal lesions				0.79
≤1	72	38	34	
≥2	36	18	18	
Bone marrow involvement				0.47
Yes/no	60/48	33/23	27/25	
Up front/relapse or refractory	48/60	23/33	25/27	0.86
Local radiation therapy before ASCT				0.22
Yes/no	16/92	6/50	10/42	
Number of prior regimens before ASCT				0.77
≤2	41	22	19	
≥3	67	34	33	
Disease status at transplantation				0.01
Complete remission	81	48	33	
Partial remission	27	8	19	
PBSCH				0.03
Numbers of CD34+ cells	3.7×10^6 (1.2–30.0)	4.1×10^6 (1.7–30.0)	3.3×10^6 (1.2–11.1)	
Conditioning regimens[c]				0.03
TBI/non-TBI	27/81	19/37	8/44	
Stem cell source				0.10
PB/BM + PB/BM	102/5/1	56/0/0	46/5/1	
CD34-selected transplantation				<0.01
Yes/no	17/91	0/56	17/35	

[a]Rituximab was used as pretransplant induction chemotherapy.
[b]*P* values were calculated by the chi-square analysis, Student's *t*-test, the Fisher exact test or two-sample Wilcoxon rank-sum (Mann–Whitney) test.
[c]No patients had rituximab given with the conditioning.
ASCT, autologous hematopoietic stem-cell transplantation; PBSCH, peripheral blood stem cell harvest; TBI, total body irradiation; PB, peripheral blood stem cells; BM, bone marrow.

anemia). Most patients had grade 3 thrombocytopenia or anemia. The rituximab usage group had a trend of the later onset of first and nadir days of DON (Figure 2b). The median duration of the DON period could not be defined in this study because most patients received outpatient care and timing of their next visit after the occurrence of DON was arbitrary by each physician's decision. During DON, 14 samples (25%) of bone marrow were obtained; 8 were hypocellular marrow and 6

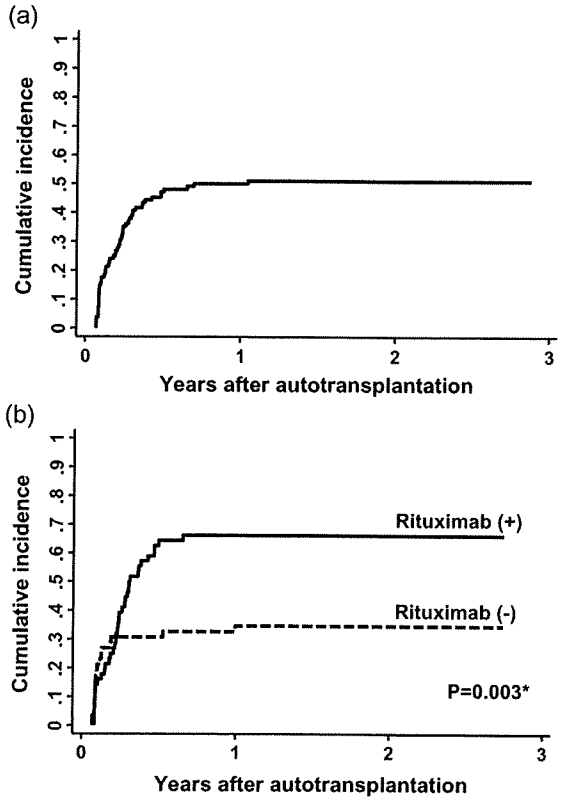

Figure 1. Cumulative incidence of DON. Cumulative incidence of DON was 50% in all patients at 1 year (a), 66% with rituximab usage group, and 33% without rituximab group. DON occurred more often in the group with rituximab usage (b). *Log-rank test. DON, delayed-onset neutropenia.

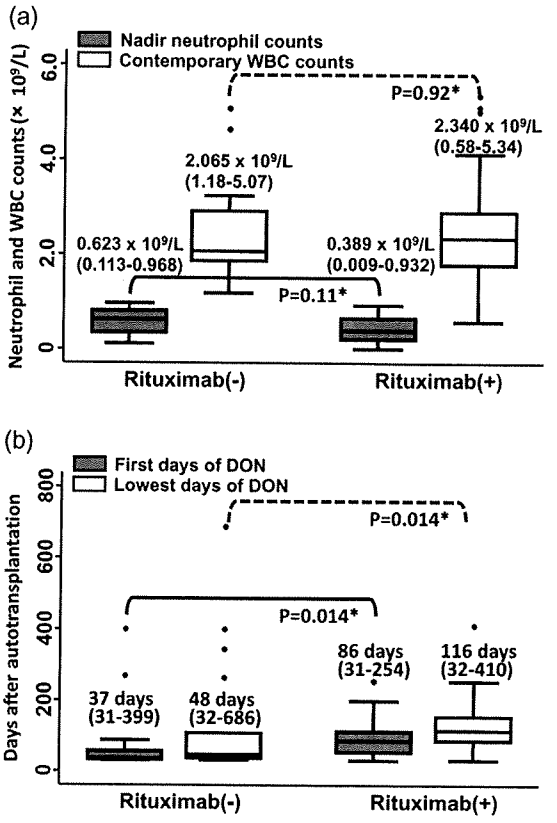

Figure 2. Nadir neutrophil counts and contemporary WBC counts (a) and first and the lowest days of DON (b) according rituximab usage. *Two-sample Wilcoxon rank-sum (Mann–Whitney) test. WBC, white blood cell; DON, delayed-onset neutropenia.

were normocellular. Some samples showed maturation arrest. B lymphocytes were depleted in about a half of the patients. An excess of T-large granular lymphocyte in bone marrow, which is indicated as a possible pathogenetic mechanisms of DON [17], was not confirmed in this study. Eleven patients (20%) received G-CSF during the period of DON.

In all period, a total of 117 events were documented (Table 2). Most infectious events (62%) were observed within a half-year, and only eight events were documented after 1.5 years. Of the 117 events, 24 infectious events were documented during the period of DON. All events were grade1–3 (most of them were grade 1–2), and early appropriate care brought to the patients rapid improvement. Neither life-threatening nor toxic death was observed. Cumulative incidence of total infectious events at 1 year was 60% (95% CI 51% to 70%), 75% (95% CI 63% to 85%), and 42% (95% CI 29% to 58%) in all patients, with, and without DON group, respectively; VZV infection was 24% (95% CI 17% to 34%), 31% (95% CI 21% to 45%), and 17% (95% CI 9% to 31%) in all patients, with, and without DON group, respectively; and upper respiratory infection was 14% (95% CI 9% to 23%), 23% (95% CI 14% to 36%), and 4% (95% CI 1.1% to 17%) in all patients, with, and without DON group, respectively (Figure 3). There had been no overt evidence of other viral-related complications such as progressive multifocal leukoencephalopathy (PML) as recently reported [18].

statistical considerations for risk factors of DON and infectious events

By stepwise method, selected factors for final validation model were rituximab usage, existence of bulky lesion, sex, pretransplantation setting, and histology. In the validation, usage of rituximab [odds ratio (OR) = 4.5, 95% CI 1.3–15.8, $P = 0.020$] and female sex (OR = 4.7, 95% CI 2.4–9.4, $P < 0.001$) were identified as a risk factor for DON (Table 3). Other factors failed to demonstrate significant impact on DON.

In univariable analyses, DON ($P = 0.001$) and age ($P = 0.072$) were identified as risk factors for total infectious events; DON ($P = 0.033$), sex ($P = 0.026$), and status at ASCT ($P = 0.076$) for VZV infection; age ($P = 0.037$), pretransplantation setting ($P = 0.103$), and number of prior chemotherapy regimens ($P = 0.053$) for HSV infection; pretransplantation setting ($P = 0.128$) and status at ASCT ($P = 0.145$) for CMV; age >60 years ($P = 0.061$), pretransplantation setting ($P = 0.126$), number of prior chemotherapy regimens ($P = 0.086$), and status at ASCT ($P = 0.100$) for fever; DON ($P = 0.016$) for upper respiratory infection; age ($P = 0.095$) for pneumonia; and DON ($P = 0.108$), rituximab usage ($P = 0.087$), number of extra nodal sites ($P = 0.015$), and

Table 2. Infectious episodes during the period with and without DON

Infections	During the period with DON ($n = 24$), n (%)	All the period ($n = 117$), n (%)
VZV	3 (13)	29 (25)
HSV	0 (0)	7 (6)
CMV[a]	1 (4)	3 (3)
Fever (>38.0°C)	4 (17)	15 (13)
Upper respiratory infections	8 (33)	26 (22)
Pneumonia[b]	2 (8)	4 (3)
Gastroenteritis	4 (17)	16 (14)
Urinary tract infections	1 (4)	12 (10)
Other infectious events[c]	1 (4)	5 (4)

[a]Positive for CMV antigenemia.
[b]Excluding interstitial lung diseases.
[c]Local bacterial infections.
DON, delayed-onset neutropenia; VZV, Varicella–zoster virus; HSV, herpes simplex virus; CMV, cytomegalovirus.

TBI ($P = 0.001$) for gastroenteritis. Multivariable analyses identified that DON was an independent risk factor for total infectious events [hazard ratio (HR) 2.3, 95% CI 1.3–3.8, $P = 0.002$] and TBI usage for gastroenteritis (HR 4.6, 95% CI 1.4–15.3, $P = 0.013$). Table 4 showed impact of DON for infectious events. DON did not affect both progression-free and overall survival (data not shown).

In subset analyses, we limited analyses to the groups of the patients receiving ASCT before ($n = 26$) and after ($n = 82$) 1999, the patients receiving CD34-selected stem-cells ($n = 17$) or not ($n = 91$), and the patients with indolent ($n = 37$) or aggressive lymphoma ($n = 71$). The groups receiving ASCT after 1999, receiving CD34-unselected stem-cells, and with indolent lymphoma demonstrated frequent occurrence of DON in rituximab usage group (log-rank test, $P < 0.01$). These groups and aggressive lymphoma showed significant impact of DON on total infectious events. The groups receiving ASCT before 1999 and CD34-selected stem-cells failed to demonstrate clinical impact of DON.

discussion

In the earlier studies of single-agent rituximab therapy in patients with relapsed low-grade B-NHL [2, 3], neutropenia was observed in 5–8% of patients between the completion of therapy and the first year of follow-up, and these neutropenia were generally transient and self-limited. Thereafter, development of neutropenia with a different pattern of onset has been reported [4, 11–14]. Nitta et al. [15] revealed that rituximab combined with chemotherapy as a primary treatment showed a 25% incidence of grade 3 neutropenia, and most patients experienced a single episode of DON. Rituximab treatment in combination with intensive or ASCT produced higher frequency of DON [6, 8, 13–15]. Moreover, DON was also documented in the patients with autoimmune diseases receiving rituximab treatment [19, 20], and the patients receiving standard chemotherapy without rituximab did not experience DON [15]. Current study also showed high incidence of DON (50%), and 61% of patients experienced ≥ 2

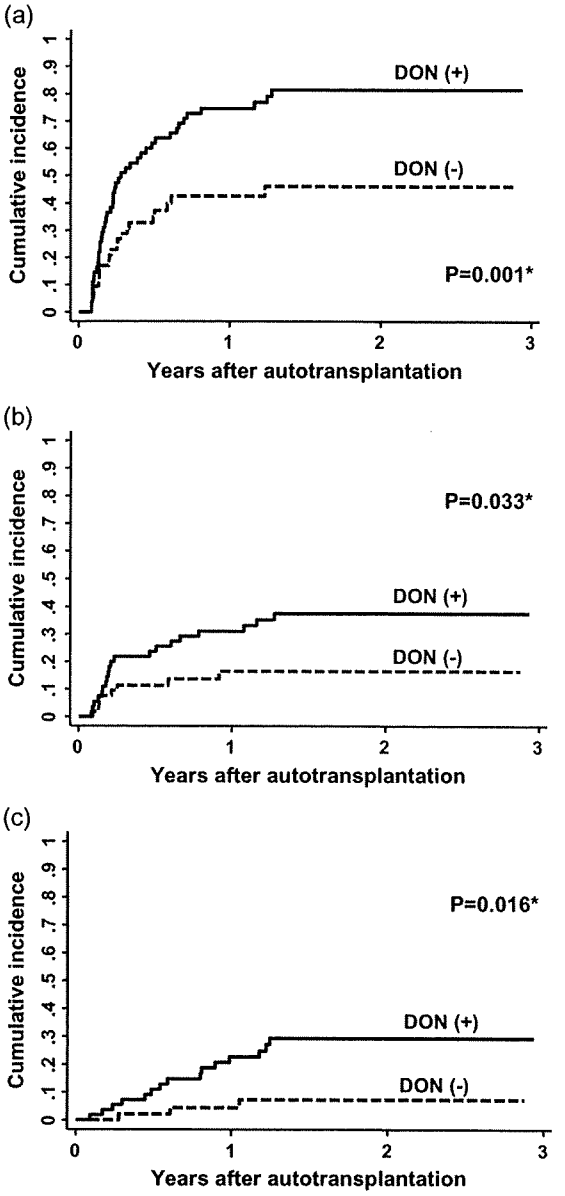

Figure 3. Cumulative incidence of infectious events. Cumulative incidence of total infectious events at 1 year was 75% and 42% with and without DON group, respectively (a); VZV infection was 31% and 17% (b); and upper respiratory infection was 23% and 4% (c). *Log-rank test. DON, delayed-onset neutropenia; VZV, Varicella–zoster virus.

episodes. Rituximab combined with myelotoxic chemotherapy could produce higher frequency of DON (66%); however, the non-rituximab patients experienced DON of a 33% incidence in our study. These results implied that DON could not be explained as a simple homogenous phenomenon.

Patients who received rituximab showed B lymphocyte depletion, and it causes significant changes of the T lymphocytes compartment [21] or a decrease in the absolute number of T lymphocytes [22]. Lymphocytes and immune system showed slow recovery after rituximab usage. Median recovery time for CD4+ T-cell counts, B-cell counts, and

Table 3. Risk factors for DON[a]

Factors	Stepwise method *P* value	Bootstrap resampling method *P* value[b]	Odds ratio (95% CI)
Rituximab usage	0.003	0.020	4.5 (1.3–15.8)
Existence of bulky lesion	0.165	Drop	–
Sex (female)	0.016	<0.001	4.7 (2.4–9.4)
Pretransplantation setting (up front)	0.153	Drop	–
Histology (indolent)	0.023	0.061	2.5 (0.96–6.4)

[a]Risk factors were explored by Cox proportional hazards model applying stepwise method.
[b]Bootstrap resampling method was applied for validation of the analyses (repeating 10 000 times with 108 sampling from original cohort).
DON, delayed-onset neutropenia; CI, confidence interval.

Table 4. Impact of DON for infectious events

Events	Univariable analysis *P* value	Multivariable analysis *P* value	HR (95% CI)
Total infectious events	0.001	0.002	2.3 (1.3–3.8)[a]
VZV infections	0.033	0.109	2.0 (0.9–4.5)[b]
Upper respiratory infection	0.016	–	4.6 (1.3–15.9)[c]
Gastroenteritis	0.108	0.407	1.7 (0.6–6.0)[d]

[a]HR adjusted for DON and age.
[b]HR adjusted for DON, sex, and status at transplant.
[c]HR adjusted for no other factors.
[d]HR adjusted for DON, rituximab usage, number of extra nodal sites, and types of containing regimens.
DON, delayed-onset neutropenia; HR, hazard ratio; VZV, Varicella–zoster virus.

immunoglobulins was a year or longer in patients receiving peri-transplantation rituximab [23]. Rituximab is thought to inhibit B lymphocyte survival and proliferation through negative regulation of canonical signaling pathways involving Akt, extracellular signal-regulated kinase (ERK), and mammalian target of rapamycin (mTOR) [24, 25]. The etiology of DON is, however, not yet clearly understood. Some factors indicated possible pathogenetic mechanisms of DON such as anti-neutrophil antibody [11, 12], T-large granular lymphocyte [17], soluble Fas-ligand [17], perturbation of stromal-derived factor 1 and granulopoiesis homeostasis during B-cell recovery [26], and an excessive B-cell depletion and recovery which were assessable by the serum level of BAFF (B-cell activation factor belonging to the tumor necrosis factor family) [27]. In this study, an excess of T-large granular lymphocyte in bone marrow was not confirmed, and all patients did not show hypoplastic marrow as previously described [28]. Considering that patients without rituximab experienced DON, rituximab usage seems not to be the sole cause. Moreover, the observation that the rituximab group had a trend of the later onset and nadir days of DON may indicate the different mechanism of DON between two groups. Further investigation is needed to clarify etiology and pathogenesis of DON.

Infectious episodes associated with DON were generally self-limited without serious infectious complication after standard chemotherapy [2–4, 11, 15]; DON, however, posed for some patients serious but not life-threatening infectious complications in the setting after ASCT [14]. In the current study, we investigated whether the clinical course was affected by the onset of DON, and detailed the infectious events not only during the period with DON, but also in the period without DON. Although DON was associated with the occurrence of infectious events after ASCT, most events were generally mild to moderate, even in the period with DON, owing to early appropriate treatment. Considering slow recovery of lymphocytes and immune system after peri-transplant rituximab usage [23], early appropriate care would be necessary to prevent infectious events from developing to severe status.

Rituximab usage itself was not identified as an independent risk factor for infectious events and many patients receiving rituximab, if experiencing DON, did not show serious viral infections in our cohort; however, severe and life-threatening viral complications have recently drawn a lot of attention on rituximab therapy. Carson et al. [18] reported 57 cases of PML after rituximab therapy in human immunodeficiency virus-negative patients from the Research on Adverse Drug Events and Reports project. PML is caused by JC polyoma virus, and the pathophysiology of rituximab-associated PML is yet fully understood, particularly with respect to the role of rituximab. It is a rare, but serious and usually fetal central nervous system infection (the case-fatality rate; 90%). Food and Drug Administration (FDA) approved the package insert of rituximab to include information regarding the increased risks of PML and other viral related complications such as hepatitis B (HB) and hepatitis C reactivation, VZV, HSV, cytomegalovirus, and parvovirus B19. More recently, FDA has declined to extend rituximab's licensed indication to include first-line therapy for rheumatoid arthritis, because of concerns related to PML. PML was not documented in our study and there is no report on association of DON with PML so far. How careful was the observation of these individuals is not yet clearly defined. Moreover, for many viral infections, anti-viral therapy may not be helpful, nor may early intervention always be clinically useful. Since rituximab is now increasingly administered for non-malignant illnesses as well [29, 30], risk-benefit considerations are important in the application of rituximab.

There were some episodes found besides our analyses. Nine HB carriers were documented. Of these, HB virus reactivation was observed in one patient, who was positive for HB envelope antigen and had received therapeutic antivirus agent before induction chemotherapy. We could not conclude definite prevalence of HB reactivation and its association with DON in this study, because most patients especially in the earlier cohort had not been tested for HB surface antibody or HB core antibody. Two patients were found to develop auto-immune diseases after ASCT [hypothyroidism and immune thrombocytopenic purpura, (ITP)]. The patient with hypothyroidism received rituximab and experienced DON; on the other hand, the patient with ITP was not affected with both. Considering rituximab usage or T-cell depletion has showed a clinically rational option to hypothyroidism [30] and its disease

prevalence was the same as a population based study, we could not make definite conclusion that the auto-immune disease was acquired through rituximab usage or occurrence of DON.

In conclusion, this study demonstrated that rituximab usage for the patients receiving ASCT was an independent risk factor of DON considering bias due to indication of rituximab treatment, and DON correlated with increased occurrence of infectious events after ASCT. Careful follow-up would be needed for the patients having an experience of DON after ASCT. The interpretation of this result may not always be comparable because of this retrospective study design. Further studies should be reported from other settings as well as some case-control type epidemiologic studies.

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disclosure

The authors indicate no potential conflicts of interest.

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ORIGINAL ARTICLE

Primary gastric diffuse large B-cell Lymphoma (DLBCL): analyses of prognostic factors and value of pretreatment FDG-PET scan

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Abstract

Objectives: We report a single institution experience with gastric diffuse large B-cell lymphoma (DLBCL) in an attempt to evaluate the roles of different treatment modalities, to assess the value of pretreatment positron emission tomography (PET) scan, and to identify potential prognostic factors. *Methods:* Among 384 patients diagnosed with DLBCL between 1995 and 2008, 75 patients had primary gastric DLBCL and were reviewed and analyzed. *Results:* The median age was 66. International prognostic index (IPI) risk was low in 52%, low-intermediate in 23%, high-intermediate in 9%, and high in 16%. Pretreatment PET scan was highly sensitive in detecting gastric lesions except stage I gastric DLBCL without detectable mass by CT or gastroscopy. As a general rule, patients with limited-stage disease were treated with three times of CHOP (with or without rituximab) and radiotherapy, and those with advanced-stage disease were treated with eight cycles of CHOP (with or without rituximab), and radiotherapy was given to residual diseases after chemotherapy. Three-year overall survival (OS) rate was 78%. Multivariate analysis revealed that low albumin, hemoglobin <12.0 g/dL, and treatment without rituximab were independently associated with shorter OS. Low albumin, hemoglobin <12.0 g/dL,and advanced stage were independently associated with shorter progression-free survival. *Conclusion:* We showed the survival benefit of rituximab and potential prognostic value of pretreatment hemoglobin and serum albumin levels in gastric DLBCL.

Key words gastric diffuse large B-cell lymphoma; serum albumin; hemoglobin; rituximab; fluorodeoxyglucose positron emission tomography

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Introduction

Gastrointestinal lymphoma is the most common form of extranodal lymphoma accounting for up to 40% of all cases, among which stomach is a most common site of involvement (1). The two most common subtypes are diffuse large B-cell lymphoma (DLBCL) and marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT). Helicobacter pylori infection plays a significant role in the development of gastric MALT lymphoma (2), and the eradication of this bacterium with antibiotics is the mainstay of the management of gastric MALT lymphoma (3). The role of Helicobacter pylori in gastric DLBCL, however, is controversial and so has been the therapeutic approach for patients with gastric DLBCL.

Gastric DLBCL has been treated with various modalities including surgery, chemotherapy, and radiotherapy alone or in combination. Historically, surgical resection of tumor was believed to be the mainstay of treatment with or without additional therapy (4, 5). Surgical resection surely provides definitive diagnosis and reduces the tumor bulk immediately. This approach, however, was questioned and several studies showed the benefit of conservative treatment with chemotherapy with or without radiotherapy, similar to other DLBCL (6–9). Surgical

intervention is thus generally believed unnecessary except for controlling complications such as major bleeding, perforation or obstruction.

Majority of studies on gastric DLBCL were reported based on studies prior to rituximab era, and the actual impact of the addition of rituximab in the management of gastric DLBCL has been evaluated only in small studies (10). Moreover, the role of fluorodeoxyglucose positron emission tomography (FDG-PET) has been assessed only in small studies (11, 12), that needs further evaluation. We herein performed a retrospective analysis of patients with gastric DLBCL diagnosed and treated in our institution. This study attempted to identify potential prognostic factors, to assess the value of pretreatment positron emission tomography (PET) scan, and to evaluate roles of different treatment modalities with focus on rituximab.

Materials and methods

Patients

We reviewed medical records of 384 patients with DLBCL newly diagnosed and treated at Aichi Cancer Center Hospital between October 1995 and August 2008. Staging evaluation for newly diagnosed patients consisted of physical examination, systemic CT scan, bone marrow aspiration and biopsy, and upper gastrointestinal endoscopy. FDG-PET also became a part of routine initial workup in 2003. Seventy-five patients (19%) had gastric involvement of DLBCL, which were all thought to be the predominant lesions of lymphoma, that is, none had 'primary lesion' other than stomach. Based on a commonly used definition of the primary gastric lymphoma, which is 'a lymphoma which present with the predominant lesion at stomach (13)', these 75 patients were analyzed in this study of primary gastric lymphoma.

Statistical analysis

The Fisher exact tests were used for the descriptive statistical analyses on categorical data. Overall survival (OS) and progression-free survival (PFS, time from diagnosis to disease progression, relapse, or death of any cause) were calculated using Kaplan-Meier method, and was compared between two groups by log-rank test. Patient characteristics were analyzed for their association with OS and PFS using Cox proportional hazard models. In these models, characteristics with P values <0.10 in the univariate analyses were included in the multivariate analyses, and a backward elimination with a P cutoff of 0.05 was used. In the final models of the multivariate analyses, any parameter could be put back into the model if the final P value <0.05. In the multivariate analyses, prognostic scores calculated by other parameters, such as international prognostic index (IPI), were not incorporated in the model unless otherwise stated. Hazard ratio (HR) and its 95% confidence interval (CI) were calculated for each parameters in the final model. All computations were performed in STATA version 9.0 (College Station, TX).

Results

Patient characteristics

Pretreatment characteristics of 75 patients with gastric DLBCL are summarized in Table 1. The median age of patients was 66 (range 21–87). None had human immunodeficiency virus (HIV-I/II) infection. As a general rule, patients with limited-stage disease were treated with three cycles of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone with or without rituximab) and radiotherapy, and those with advanced-stage disease were treated with eight cycles of CHOP (with or without rituximab), and radiotherapy was given to residual diseases after chemotherapy. Gastrectomy was performed as a part of diagnostic process or to manage the complication such as bleeding or obstruction. Actual treatment strategies given are summarized in Table 1.

There was no evidence of disease in 62 patients (83%) after planned initial treatments. Patients experiencing refractory or relapsed disease after initial treatment were treated with salvage chemotherapy containing high-dose cytarabine and etoposide (14, 15). After the year 1996, patients aged 65 or younger with age-adjusted IPI score (scored by stage ≥3, elevated serum lactate dehydrogenase level (LDH), number of extranodal involvement ≥2) of 2 or 3 were generally offered an option of upfront autologous stem cell transplantation (ASCT) if patients achieve CR or partial response (PR) after induction therapy, and three such patients underwent ASCT as a part of primary treatment. Another two patients underwent ASCT in the salvage settings.

FDG-PET sensitivity for gastric DLBCL

We next assessed the role of pretreatment PET scan for staging, as CT scan is generally not sensitive in detecting gastrointestinal lesions. In 52 patients (69%), gastroscopy that was performed for screening purposes or digestive symptoms led to the diagnosis of lymphoma (group A). In 17 patients (23%), gastroscopy performed for the staging purpose for proven lymphoma of other site revealed gastric DLBCL, (group B). The diagnostic process was unclear in six patients (8%) because evaluation was initiated in other institutions.

Table 1 Patient characteristics

Parameters		*n* (%)
All		75
Age (years)	≤60	27 (36)
	>60	48 (64)
Sex	Male	43 (57)
	Female	32 (43)
Ann Arbor Stage (26)	I/II	50 (67)
	III/IV	25 (33)
Lugano Stage (27)	I/II1	35 (47)
	II2/IV	40 (53)
PS	0/1	60 (80)
	≥2	15 (20)
LDH	Normal	45 (60)
	High	30 (40)
B symptoms	Absent	62 (83)
	Present	13 (17)
IPI risk group (28)	Low	39 (52)
	Low intermediate	17 (23)
	High intermediate	7 (9)
	High	12 (16)
Serum albumin	Lower than LLN	32 (43)
	Normal	42 (57)
Hemoglobin	<12 mg/dL	25 (34)
	≥12 mg/dL	49 (66)
Absolute lymphocyte count	$<1.0 \times 10^9$/L	30 (41)
	$\geq 1.0 \times 10^9$/L	44 (59)
sIL2R	<1000 U/ml	38 (54)
	≥1000 U/ml	33 (46)
B2-microglobulin	<2 mg/L	19 (44)
	≥2 mg/L	24 (56)
Histology: Low-grade component	Absent	64 (85)
	Present	11 (15)
Immunophenotype by Hans algorithm (29)	GCB	17 (49)
	Non-GCB	18 (51)
BCL2	Positive	32 (59)
	Negative	22 (41)
Treatment		
Limited disease (stage I/II1, *n* = 35)		
Surgical resection (*n* = 8)	Surgery alone	2
	Surgery + CHOP	5
	Surgery + R-CHOP	1
No surgery (*n* = 27)	CHOP alone	1
	R-CHOP alone	1
	CHOP + Radiotherapy	6
	R-CHOP + Radiotherapy	16
	Radiotherapy alone	3
Advanced disease (stage II2/IV, *n* = 40)	CHOP alone	7
	CHOP + Surgical resection	4
	CHOP + Radiotherapy	3
	R-CHOP alone	18
	R-CHOP + Surgical resection	2
	R-CHOP + Radiotherapy	6
ASCT	As a primary treatment	3
	In salvage settings	2
	No transplant	70
NED		83%
Three-year OS rate		78%
Three-year PFS rate		70%

Abbreviations: PS, Eastern Cooperative Oncology Group Performance Status; LDH, serum lactate dehydrogenase level; B symptoms, presence of at least one of the followings: night sweat, weight loss >10% over 6 months, and recurrent fever >38.3°C); IPI, International Prognostic Index; sIL2R, serum soluble interleukin-2 receptor level; GCB, germinal center B-cell-like; ASCT, autologous stem cell transplant; OS, overall survival; PFS, progression-free survival; LLN, lower limit of normal range; NED, no evidence of disease at the end of planned initial therapy.
Numbers may not add up to 75 for some characteristics because of unavailable information.

Twenty-two patients in group A underwent pretreatment PET scans. Gastric lymphoma was not detected by PET scan in five patients (sensitivity 77%). All these five had stage I disease. None of five had detectable mass either by CT scan or gastrointestinal endoscopy, but all were diagnosed with gastric DLBCL from biopsies of gastric erosive mucosa. Eleven patients in group B underwent pretreatment PET scan, which all detected gastric lesions (sensitivity: 100%).

Overall survival and prognostic value of albumin and hemoglobin

The median follow-up duration was 32 months, and the estimated 3-year OS rate was 78%. Univariate analysis in all patients revealed that low albumin, elevated LDH, PS ≥ 2, stage III/IV (AnnArbor staging) and stage II2/IV (Lugano staging for gastrointestinal lymphoma), presence of B symptoms, higher IPI risk group, high β2-microglobulin, treatment without rituximab were significantly associated with shorter OS duration.

Those with gastric DLBCL with low-grade component tended to have longer OS than those without low-grade component, although the difference was not statistically significant (P = 0.07, Fig. 1). There was no significant difference in OS duration between those with germinal

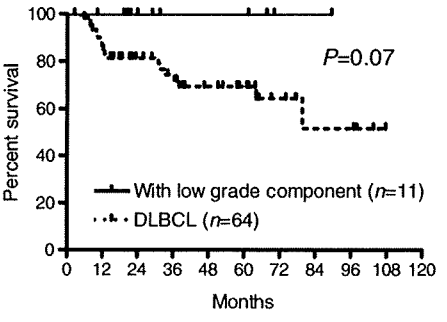

Figure 1 Overall survival according to histology

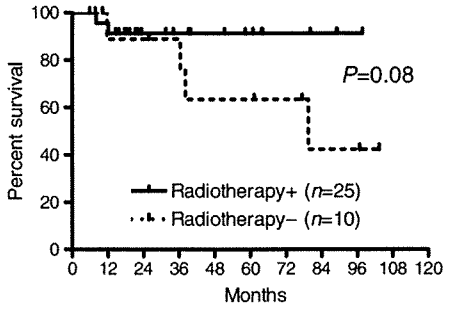

Figure 2 Overall survival in patients with limited-stage disease treated with or without radiotherapy

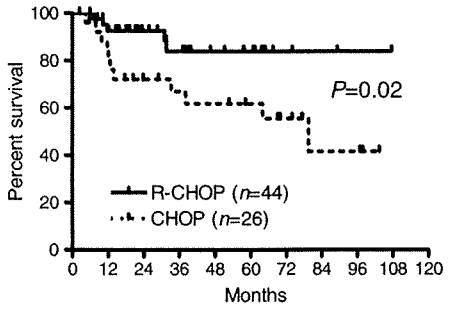

Figure 3 Overall survival in patients treated with CHOP or R-CHOP. Five patients who did not receive systemic therapy were excluded

Table 2 The result of multivariate analyses for overall survival and progression-free survival

	Hazard ratio (95% CI)	*P*- value
Overall survival		
Low hemoglobin (<12.0 g/dL)	4.32(1.58–11.8)	0.004
Low albumin (<LLN)	3.07(1.07–8.80)	0.037
Treatment without rituximab	2.70(1.00–7.25)	0.049
Progression free survival		
Low hemoglobin (<12.0 g/dL)	3.52(1.47–8.45)	0.005
Low albumin (<LLN)	2.52(1.00–6.35)	0.049
Advanced stage (Stage III/IV)	2.85(1.23–6.62)	0.015

Abbreviations: lower limit of normal range; CI, confidence interval.

center B-cell-like (GCB) and those with non-GCB (P = 0.58). In patients with limited-stage disease, those receiving radiation therapy after chemotherapy tended to have longer OS than those who did not receive radiotherapy (P = 0.08, Fig. 2). The OS duration was longer in patients who received R-CHOP than in those received CHOP (P = 0.02, Fig. 3).

Multivariate analysis for OS in all patients revealed that low hemoglobin (HR 4.32 [1.58–11.83], P = 0.004), low albumin (HR 3.07 [1.07–8.80], P = 0.037), and treatment without rituximab (HR 2.70 [1.00–7.25], P = 0.049) were independently associated with shorter OS duration (Table 2).

Progression-free survival

The estimated 3-year progression-free survival rate was 70%. Univariate analysis revealed that low albumin, elevated LDH, PS≥2, stage III/IV (AnnArbor staging) and stage II2/IV (Lugano staging for gastrointestinal lymphoma), presence of B symptoms, higher IPI risk group, BCL2 expression, high sIL-2R, treatment without radiotherapy, and treatment without rituximab were significantly associated with shorter PFS duration. By multivariate analysis, low hemoglobin [HR 3.52 (1.47–8.45), P = 0.005], low albumin [HR 2.52 (1.00–6.35), P = 0.049], and advanced stage according to AnnArbor staging [HR 2.85 (1.23–6.62), P = 0.015] were independently associated with shorter PFS duration (Table.2).

Discussion

The management of gastric DLBCL had been controversial. In the recent years, conservative approaches such as radiation therapy and/or chemotherapy are generally favored as opposed to surgical resection of tumor in the management of primary gastrointestinal lymphomas, because at least in part of morbidities associated with gastrectomy (16). In addition, resection of lesions of DLBCL alone is associated with high rate of relapse in general such that systemic chemotherapy should be given even after resection of all visible lesions (17). This analysis may be truly biased by the fact that some received no chemotherapy because of underlying diseases, which by itself can be associated with poor prognosis. Yet, it is believed that systemic therapy plays a critical role in the management of gastric DLBCL, even in those with limited diseases.

Low serum albumin level and low hemoglobin level were identified as independent prognostic factors in our study. Serum albumin level is an established critical prognostic factor in patients with Hodgkin lymphoma (18). Several small studies have shown its potential prognostic value also in other lymphomas (19–21). Serum albumin is a parameter of gross nutritional status that could be influenced by abnormal metabolic conditions caused by malignancies with or without gastrointestinal tract. We believe this finding is interesting and warrants further investigation in larger group of patients. Low hemoglobin level also is an important poor prognostic parameter in Hodgkin lymphoma (18) as well as other lymphomas (19, 22). Anemia can be as a result of iron deficiency associated with gastric bleeding, bone marrow suppression caused by direct lymphoma involvement,

'anemia of chronic disease' and others. Actual causes of anemia, however, could be multifactorial and difficult to clearly identify in clinical practice. Nonetheless, it is a readily available parameter at diagnosis, which carries prognostic importance.

The role of radiotherapy had been established in the management of limited-stage DLBCL in the prerituximab era (17, 23). In the era of rituximab, however, radiation therapy may not be preferred as much considering the long-term toxicity of radiation exposure (24). Our study showed survival benefit with radiotherapy when analysis was performed including all patients with limited-stage gastric DLBCL. When analysis was performed separately according to the use of rituximab, however, the benefit was not statistically significant. Our study included limited number of patients treated with rituximab, and thus, further investigation of the role of radiation therapy in gastric DLBCL in the era of rituximab is of extreme importance.

Rituximab has an established role in the management of DLBCL. But information on the role of rituximab specifically for gastric DLBCL is limited. Wohrer et al. (10) reported an excellent result of R-CHOP, where fifteen patients with limited-stage gastric DLBCL were treated with R-CHOP resulting in CR rate of 87%. Our study, although retrospective in nature and with limited number of patients, confirmed the survival benefit of treatment with rituximab in this small subset of patients with gastric DLBCL. It is not a surprising finding but clinically important information, particularly because essentially all patients with CD20 positive DLBCL (of any subtype) now receive rituximab.

Low-grade component was observed along with DLBCL in 11 patients (15%) in our study. These patients generally had favorable prognosis compared to those with 'de novo' DLBCL. It is not technically feasible to definitely determine the 'absence' of low-grade component in gastric DLBCL without removing the whole tumor. Yet, our data suggests that detection of low-grade component in DLBCL may have favorable prognostic impact. On the other hand, there was no difference in survival between patients with GCB subtype or non-GCB subtype in our study of gastric DLBCL. Given the small number of patients in our study, larger studies are needed to validate the impact of histopathological findings.

Our study suggested that PET scan may not be sensitive in detecting gastric DLBCL presenting without detectable masses by CT or gastroscopy. On the other hand, those in Group B, that is, gastric lymphoma were detected during the staging process, all had positive PET scans. Gastroscopy has been a part of routine staging work up in our institution. In the era of PET scan (11, 25), however, routine gastroscopy probably may not have a major role for staging. Our analysis is again based on the limited number of patients and thus needs to be evaluated in larger scale studies.

In conclusion, we showed potential prognostic value of hemoglobin and albumin level in patients with primary gastric DLBCL. Larger-scale studies are needed to validate our findings, and more data is needed to determine the value of pretreatment PET scan in this patient group.

Conflict-of-interest Disclosure

None.

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ORIGINAL ARTICLE

Low absolute lymphocyte count is a poor prognostic marker in patients with diffuse large B-cell lymphoma and suggests patients' survival benefit from rituximab

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Abstract

Objectives: To evaluate the prognostic value of absolute lymphocyte count (ALC) at diagnosis in patients with diffuse large B-cell lymphoma (DLBCL). *Methods:* In a large cohort of patients with DLBCL treated with CHOP (n = 119) or RCHOP (n = 102) in our institution, we evaluated the prognostic value of ALC at diagnosis with regards to treatment response, overall (OS) and progression-free survival (PFS). Use of rituximab, all International Prognostic Index (IPI) determinants, β2microglobulin level, presence of B symptoms or bulky disease, and ALC were evaluated. *Results:* Low ALC ($<1.0 \times 10^9$/L) was associated with advanced stage, performance status ≥2, elevated lactate dehydrogenase, number of extranodal involvement ≥2, B symptoms, elevated β2microglobulin and higher IPI risk group. Low ALC was associated with lower CR rate by univariate analysis (odds ratio = 3.29, P = 0.024) but not by multivariate analysis. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter OS [hazard ratio (HR) = 2.89, $P < 0.001$] and PFS (HR = 2.91, $P < 0.001$). Multivariate analysis revealed that low ALC was associated with shorter OS (HR = 2.51, P = 0.003) and PFS (HR = 2.72, $P < 0.001$), independent of above-mentioned parameters. Subclass analyses revealed that the use of rituximab improves OS in patients with low ALC (HR = 0.42, P = 0.05) but not in those with high ALC (HR = 0.83, P = 0.71). This observation was most obvious in patients with higher IPI score. *Conclusion:* Low ALC is a poor prognostic marker in patients with DLBCL and suggests patients' survival benefit from rituximab.

Key words absolute lymphocyte count; diffuse large B-cell lymphoma; prognostic factor; rituximab

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Prognostication of patients with diffuse large B-cell lymphoma (DLBCL) is important in determining optimal treatment approaches. Numbers of prognostic factors have been studied, but some require expensive molecular testing and thus not clinically applicable. Inexpensive and readily available prognostic factors are practical and helpful.

Low absolute lymphocyte count (ALC) at diagnosis is associated with poor prognosis in patients with advanced Hodgkin lymphoma (1) as well as follicular lymphoma (2). A recent preliminary study with short follow-up duration also suggested a potential prognostic value of ALC in DLBCL (3). While International Prognostic Index (IPI) is currently the most valuable prognostic indicator in patients with aggressive lymphoma, ALC was not included in the parameters analyzed (4). We performed a retrospective study evaluating the prognostic value of low ALC using our large cohort of patients with DLBCL, about half treated with CHOP and the rest with RCHOP.

Patients and methods

This retrospective study was approved by the institutional review board. We reviewed 221 consecutive newly

diagnosed patients with non-HIV-associated DLBCL who were treated with CHOP (n = 119; before approval of rituximab) or RCHOP (n = 102; after approval) based therapy at Aichi Cancer Center Hospital between January 1999 and January 2007. Age (≤60 or >60), performance status (PS, ≤1 or ≥2), B symptoms (present or absent), stage (≤2 or ≥3), number of extranodal involvement (≤1 or ≥2), bulky disease (largest diameter of the disease ≥10 cm, present or absent) serum lactate dehydrogenase (LDH) levels (normal or elevated), ALC at diagnosis, IPI group (scored from 0 to 5 by age >60, stage ≥3, PS ≥2, LDH higher than upper limit of normal range and number of extranodal involvement ≥2, and risk groups were classified as low by score 0/1, low-intermediate by score 2, high-intermediate by score 3 and high by score 4/5), initial treatment (CHOP or RCHOP) were collected and incorporated as potential prognostic factors in various analyses. Serum β2microglobulin level was collected if available but excluded from the survival analyses because of many missing data.

The Fisher exact tests were used for the descriptive statistical analyses on categorical data. Overall survival (OS) and progression free survival (PFS, time from diagnosis to disease progression, relapse or death of any cause) were calculated using Kaplan–Meier method (5) and was compared between two groups by log-rank test. Logistic regression models were used to evaluate the associations between multiple characteristics and complete response (CR). Patient characteristics were also analyzed for their association with PFS and OS using Cox proportional hazard models. In this model, characteristics with P-values <0.10 in the univariate analyses were included in the multivariate analyses, and a backward elimination with a P-cutoff of 0.05 was used. All computations were performed in STATA version 9.0 (StataCorp, College Station, TX, USA).

Results

Patient characteristics

Patient characteristics are summarized in Table 1. There was no significant difference in baseline characteristics between CHOP and RCHOP group. In patients with early-stage non-bulky disease, involved field radiation therapy was performed following three courses of CHOP (n = 37) or RCHOP (n = 38) therapy. Patients younger than 65 with age-adjusted IPI score of 2 or 3 were generally offered an option of upfront autologous stem cell transplantation after induction therapy, and 20 such patients (11 after CHOP and nine after RCHOP) underwent this treatment.

The median value of ALC of entire population was 1.20×10^9/L (range 0.10–4.64 $\times 10^9$/L). ALC was significantly higher in IPI low risk (median ALC 1.49×10^9/L), and the values were not significantly different among low-intermediate (median 0.97×10^9/L), high-intermediate (median 0.93×10^9/L) and high-risk (median 0.83×10^9/L) groups (Fig. 1). Low ALC [$< 1.2 \times 10^9$/L (median value)] was associated with advanced stage, PS ≥2, elevated LDH, number of extranodal involvement ≥2, B symptoms, elevated β2microglobulin and higher IPI risk group. Using different cutoff value of ALC (0.8, 1.0 and 1.4×10^9/L) revealed essentially the same result (data using the cutoff value of 1.0×10^9/L are shown in Table 1).

Treatment response

Response to initial treatment was evaluable in 210 patients, among whom CR rate was 91.9%. CR rates in patients with low and high ALC after CHOP were 85.0% (34/40) and 97.3% (72/74), respectively (P = 0.021). Those after RCHOP were 87.5% (35/40) and 92.9% (52/56), respectively (P = 0.483). Univariate analysis using logistic regression model for the chance of achieving CR revealed that elevated LDH, PS ≥2, number of extranodal involvement ≥2 and presence of B symptoms were significantly associated with lower chance of achieving CR. Low ALC [$< 1.2 \times 10^9$/L (median value)] was not significantly associated with low CR rate {odds ratio of low ALC ($< 1.2 \times 10^9$/L) = 2.63 [95% confidence interval (CI) 0.894–7.77], P = 0.079}. Other cutoff values (0.8, 1.0 and 1.4×10^9/L) were also tested in association with CR rate, and the association was significant when cutoff value of 1.0×10^9/L was used [odds ratio of low ALC ($< 1.0 \times 10^9$/L) for low CR rate = 3.29 (95% CI 1.17–9.30), P = 0.024]. The cutoff value of 1.0×10^9/L was also found to be optimal in the survival analyses as shown later. Higher IPI risk group was also associated with lower CR rate [RR = 1.68 (1.11–2.55), P = 0.014]. Multivariate analysis revealed that only PS ≥2 [RR = 5.47 (1.87–16.0), P = 0.002] and elevated LDH [RR = 4.66 (1.25–17.3), P = 0.022] were independently associated with lower CR rate.

Overall survival

The median follow-up duration in the entire population, CHOP and RCHOP groups were 47, 67 and 29 months, respectively. Two-year OS rates in CHOP and RCHOP groups were 82.1 ± 3.6% and 87.0 ± 3.7%, respectively. The Kaplan–Meier OS estimate curves were first plotted according to ALC groups (<0.61, 0.61–0.80, 0.81–1.00, 1.01–1.20, 1.21–1.40, 1.41–1.60 and $> 1.60 \times 10^9$/L) to find the optimal cutoff value to define low and high ALC groups. This revealed that OS was generally longer in patients with higher ALC and curves

Table 1 Patient characteristics

Parameters	*n* (total 221)	ALC < 1.0×10^9/L	*P*-value	Rituximab	*P*-value
All	221	86		102	
Age (yr)					
≤60	106	37	0.270	47	0.686
>60	115	49		55	
Stage					
1/2	136	35	<0.001	62	0.890
3/4	85	51		40	
PS					
0/1	184	62	0.001	85	1.000
≥2	37	24		17	
LDH					
Normal	119	29	<0.001	51	0.344
High	102	57		51	
Number of extranodal involvement					
0/1	177	58	<0.001	83	0.736
≥2	44	28		19	
B symptoms					
Absent	188	65	0.003	84	0.346
Present	33	21		18	
IPI					
Low	117	26	<0.001	50	0.508
Low-intermediate	37	19		20	
High-intermediate	36	21		19	
High	31	20		13	
Bulky disease (≥10 cm)					
No	202	79	1.000	90	0.150
Yes	19	7		12	
Serum β2microglobulin					
<3.0 mg/dL	98	33	0.036	43	0.683
≥3.0 mg/dL	32	18		16	
NA	91	35		43	
Treatment					
CHOP	119	42	0.269	0	–
RCHOP	102	44		102	
ALC					
<1.0×10^9/L	86	86	–	44	0.269
≥1.0×10^9/L	135	0		58	

PS, Eastern Cooperative Oncology Group Performance Status; LDH, serum lactate dehydrogenase level; B symptoms, presence of at least one of the followings – night sweat, weight loss >10% over 6 months and recurrent fever >38.3°C; IPI, International Prognostic Index; ALC, absolute lymphocyte count; NA, not available.
P-values were calculated by Fisher exact test.

were grossly separated at a cutoff value of 1.0×10^9/L (data not shown). To confirm the optimal cutoff values for determining 'low ALC', we next performed sensitivity analysis, where among candidate cutoff values of 0.8, 0.9, 1.0, 1.1, 1.2, 1.3 and 1.4×10^9/L, the maximal hazard ratio (HR) was produced with the cutoff value of 1.0×10^9/L [HR = 2.89 (95% CI 1.61–5.17)]. Low ALC was thus defined to be $< 1.0 \times 10^9$/L for further survival analyses. The Kaplan–Meier OS estimate curves, calculated according to treatment (CHOP and RCHOP) and ALC (high and low) are shown in Fig. 2A. In CHOP group, 2-yr OS rates in patients with high and low ALC were 90.7 ± 3.6% and 66.5 ± 7.3%, respectively. Those in RCHOP group were 92.1 ± 3.8% and 79.8 ± 7.0%, respectively. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter OS duration in the entire population [HR = 2.89 (1.61–5.17), $P < 0.001$] or in CHOP group [HR = 3.61 (1.81–7.20), $P < 0.001$] but the difference was not significant in RCHOP group [HR = 1.78 (0.599–5.32), $P = 0.298$].

Multivariate analysis for OS incorporating all the characteristics except IPI risk group revealed that PS ≥2 [HR = 3.34 (1.82–6.15), $P < 0.001$], low ALC [HR = 2.51 (1.38–4.58), $P = 0.003$] were independently associated with shorter OS. In this model, rituximab was forced in the analysis [HR = 0.530 (0.276–1.02), $P = 0.057$]. Furthermore, when IPI risk group (analyzed

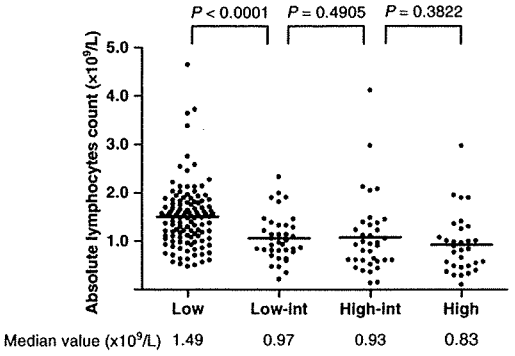

Figure 1 Absolute lymphocyte count according to IPI risk group.) *P*-values were calculated by non-parametric non-paired *t*-test (Man-Whitney test).

as a linear parameter) was incorporated instead of five IPI factors (i.e. age, PS, LDH, stage and number of extranodal involvement were omitted), low ALC was associated with shorter OS [HR = 2.11 (1.12–3.95), P = 0.019], along with IPI group [HR 1.50 (1.16–1.92), P = 0.002], where rituximab was again forced in the model [HR = 0.531 (0.278–1.02), P = 0.056, Table 2]. Removing rituximab from the final model showed the similar result for both analyses. Analyzing IPI risk group as a categorical parameter also showed essentially the same result [HR of low ALC = 2.05 (1.09–3.86), P = 0.026, Table 2].

Given that the baseline patient characteristics were similar in CHOP and RCHOP group (Table 1), OS was next compared between CHOP and RCHOP groups, according to ALC group. Use of rituximab was associated with longer OS in low ALC group [HR = 0.42 (0.18–1.00), P = 0.05] but not in high ALC group [HR = 0.83 (0.31–2.21), P = 0.71]. This suggests that the prognostic significance of ALC became smaller in the era of rituximab, as shown earlier, because the absolute survival benefit from rituximab is larger in low ALC group than in high ALC group (Fig. 2A). To further evaluate the significance of ALC and rituximab use, we next performed subgroup analyses of OS based on IPI risk group (Fig. 2B,C). In this analyses, we defined two IPI risk group [score '0–1' (n = 117) and '2–5' (n = 104)] because of significantly higher ALC distribution only in '0–1' group (Fig. 1), and limited number of patients in each low-intermediate, high-intermediate and high-risk group. The use of rituximab in patients with IPI '2–5' group with low ALC was associated with longer OS [HR = 0.35 (0.12–0.98), P = 0.045], but not in IPI '2–5' with high ALC [HR = 1.02 (0.33–3.13), P = 0.978], or in IPI '0–1' with low ALC [HR = 0.83 (0.15–4.44), P = 0.824] or in IPI '0–1' with high ALC [HR = 0.42 (0.05–3.67), P = 0.432].

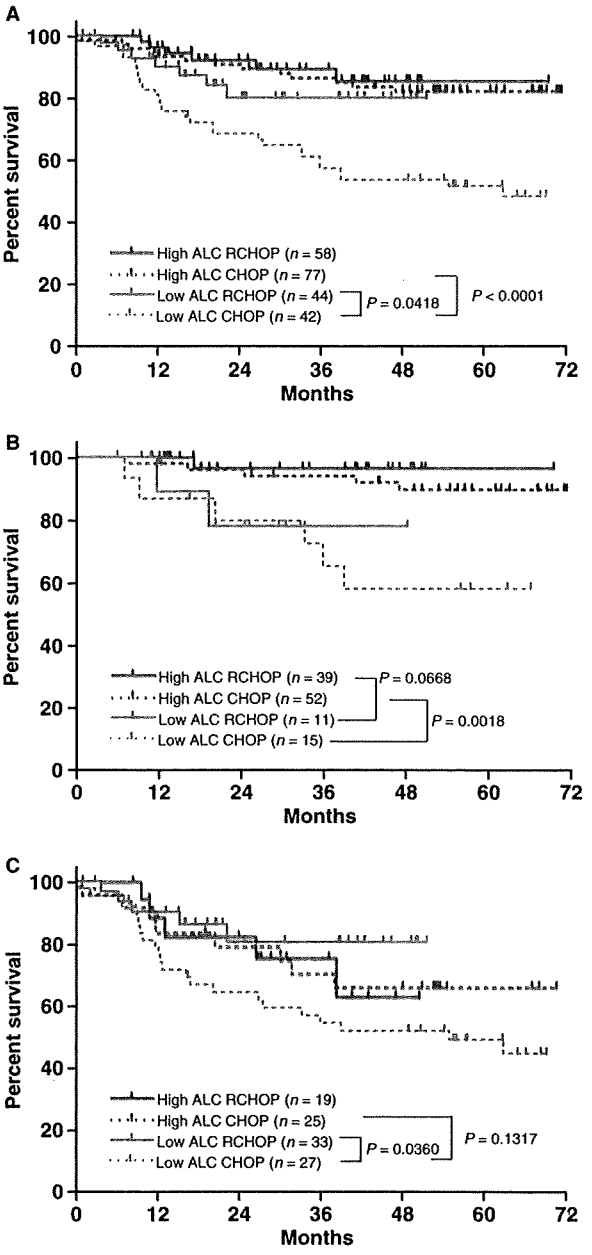

Figure 2 Overall survival according to absolute lymphocyte count and use of rituximab.) (A) All patients; (B) IPI score 0–1; (C) IPI score 2-5. *P*-values were calculated by Log-rank test. *P*-value for any survival comparison was >0.1 if not shown.

Progression free survival

We also performed analyses for PFS. Two-year PFS rates in CHOP group and RCHOP group were 72.8 ± 4.1% and 81.2 ± 4.2%, respectively. In CHOP group, 2-yr PFS rates in high and low ALC groups were 82.8 ± 4.3% and 54.6 ± 7.7%, respectively. In RCHOP group, those were

Table 2 The result of multivariate analyses for OS and PFS when IPI group was analyzed either as a linear parameter or a categorical parameter.

	Hazard ratio	95% CI	*P*-value
For OS			
Low ALC (<1.0 × 10^9/L)	2.11	1.12–3.95	0.019
IPI as a linear parameter	1.50	1.16–1.92	0.002
Rituximab (forced in the model)	0.531	0.278–1.02	0.056
For OS			
Low ALC (<1.0 × 10^9/L)	2.05	1.09–3.86	0.026
IPI			
Low-intermediate vs. low	1.93	0.831–4.49	0.126
High-intermediate vs. low	1.29	0.511–3.27	0.588
High vs. low	3.26	1.19–7.11	0.003
B symptoms	2.37	1.16–4.83	0.018
Rituximab	0.471	0.243–0.915	0.026
For PFS			
Low ALC (<1.0 × 10^9/L)	2.17	1.25–3.76	0.006
IPI as a linear parameter	1.53	1.22–1.91	<0.001
Rituximab	0.452	0.255–0.801	0.007
For PFS			
Low ALC (<1.0 × 10^9/L)	2.22	1.28–3.87	0.005
IPI			
Low-intermediate vs. low	1.52	0.703–3.28	0.287
High-intermediate vs. low	1.72	0.813–3.62	0.156
High vs. low	3.80	1.95–7.44	<0.001
Rituximab	0.457	0.258–0.810	0.007

84.0 ± 5.3% and 77.8 ± 6.6%, respectively. By univariate analysis using Cox proportional hazard model, low ALC was associated with shorter EFS duration in the entire population [HR = 2.91 (1.75–4.86), $P < 0.001$] or in CHOP group [HR = 3.90 (2.12–7.16), $P < 0.001$] but the difference was not significant in RCHOP group [HR = 1.68 (0.647–4.35), $P = 0.287$]. Multivariate analysis for PFS incorporating all characteristics except IPI group revealed that PS ≥2 [HR = 3.40 (1.98–5.83), $P < 0.001$], low ALC [HR = 2.72 (1.61–4.60), $P < 0.001$] and rituximab [HR = 0.433 (0.242–0.772), $P = 0.005$] were independently associated with shorter PFS. When IPI group as a linear parameter was incorporated instead of five IPI factors, low ALC was again associated with shorter PFS [HR = 2.17 (1.25–3.76), $P = 0.006$], along with IPI group [HR 1.53 (1.22–1.91), $P < 0.001$] and rituximab [HR = 0.452 (0.255–0.801), $P = 0.007$, Table 2]. Analyzing IPI risk group as a categorical parameter also showed similar result [HR of low ALC = 2.22 (1.28–3.87), $P = 0.005$, Table 2].

Discussion

ALC is an objective and reproducible test result, which can be obtained by basic laboratory equipment. Our study demonstrated that low ALC is a poor prognostic factor with regards to OS and PFS. Such prognostic value of ALC is in agree with other recently published studies (3, 6, 7). Although the actual mechanisms of this association between low ALC and poor prognosis is unclear, possibilities include: (i) low ALC may be associated with already immunosuppressed condition, suggesting that the host tends to have an inadequate immunological reaction; (ii) low ALC may be a consequence of lympholytic cytokines produced by lymphoma cells, and such lymphoma may already have a resistant character by itself; or (iii) the combination of these two or other.

The prognostic value of ALC was most remarkable in patients treated with CHOP without rituximab. The difference of prognostic impact between CHOP and RCHOP groups is largely because of the improvement of survival by rituximab in patients with low ALC. Particularly in the group of IPI score 2–5, treatment with rituximab significantly improved survival of patients with low ALC, but not significantly that of patients with high ALC. Analogy of this prognostic value of low ALC is that of expression of BCL2, which was a significant poor prognostic indicator before the emergence of rituximab but not in the era of rituximab (8).

Obvious limitation of this comparison is that salvage regimens might or might not have contained rituximab in relapsed patients in CHOP group (although this would not affect OS), and that the patients were not randomized (although characteristics shown in Table 1 were similar in the two groups). Although not using rituximab in addition to CHOP in any patients with DLBCL may not be justifiable given the little toxicity and significant potential benefit (9, 10), it should be noted that the absolute survival benefit is likely larger in patients with low ALC than in those with high ALC, in the era of multiple target therapy agents (currently approved or not) which may lead to expanding costs with significant impact on the economy.

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Conflict of interest

None.

Authors' contributions

All authors contributed to the patient care and data collection. Y. O. designed the study, analyzed data and wrote the paper. K. Y. analyzed the data and edited the paper. H. K. and Y. Kuwatsuka edited the paper. H. T. and Y. Kagami reviewed the paper. Y. M. supervised the patient care and edited the paper.

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