Original Article

Serum IgG and lymphocyte counts are useful for the early detection of infection in patients receiving bendamustine-rituximab therapy

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Bendamustine-rituximab (BR) therapy has been established as a highly effective regimen for indolent non-Hodgkin lymphoma (NHL). However, patients who receive BR therapy exhibit persistent hypogammaglobulinemia and lymphopenia, resulting in an increased incidence of infections. As a sustained immunosuppressive state is a risk factor for infections, early predictive biomarkers for infections related to BR therapy need to be identified. We retrospectively analyzed 61 patients with indolent NHL who were followed up for 2 years after the end of BR therapy. Progression-free survival was significantly influenced by the incidence of infections. Patients with infections related to BR therapy exhibited persistent hypogammaglobulinemia and lymphopenia. In addition, we determined the cutoff values of serum IgG values and lymphocyte counts for infections using receiver operating characteristic curve analysis. Minimum serum IgG and lymphocyte counts at the first BR treatment cycle were significantly associated with the incidence of infections during and after BR treatment. Furthermore, the development of skin reactions during BR therapy was significantly associated with the incidence of infections after BR therapy. Our study suggested that these values and symptom are predictive biomarkers for infections related to BR therapy. Based on these findings, better management of indolent NHL patients will be possible.

Keywords: bendamustine, rituximab, lymphopenia, IgG

INTRODUCTION

Bendamustine is an alkylating agent that contains a nitrogen mustard group and a unique purine-like benzimidazole ring,[1,2] with combined alkylating and antimetabolite properties.[3-6] Therefore, bendamustine has low cross-resistance with other alkylating agents. It is widely used as a first-line therapy for indolent non-Hodgkin lymphoma (NHL), including follicular lymphoma (FL), marginal zone lymphoma (MZL), and mantle cell lymphoma (MCL). In particular, bendamustine-rituximab (BR) therapy has been established as a highly effective first-line combination therapy for indolent NHL.[7] However, BR therapy causes several characteristic toxicities. Persistent hypogammaglobulinemia[8] and lymphopenia[7,9] related to BR therapy are closely associated with the incidence of infections. Bendamustine is highly toxic to T cells[2,9] and rituximab, a first-in-class anti-CD20 antibody, also suppresses the functions of B cells. Therefore, BR therapy suppresses both T cell and B cell functions, leading to susceptibility to bacterial and viral infections. In addition, skin toxicity is a well-known adverse event related to bendamustine treatment,[7,10] in which rash develops approximately 10 days after starting BR therapy.[11] The development of rash is also associated with immunosuppression, especially CD4+ T cell depletion.[10,12]

The clinical impact of infections related to bendamustine therapy remains to be elucidated. In the COVID-19 era, more caution is needed when administering chemotherapy with strong immunosuppressive agents such as BR therapy. Thus, we need to clarify the clinical significance of infections related to BR therapy and identify the clinical indicators that predict susceptibility to infections in patients who receive BR therapy. We therefore conducted a retrospective review of patients with indolent NHL who received BR therapy.

MATERIALS AND METHODS

Study design and patients

We retrospectively reviewed 61 consecutive patients who received BR therapy for indolent NHL, including FL and MCL, and MZL, including mucosa-associated lymphoid tissue (MALT) lymphoma, at Aizu Medical Center of Fukushima Medical University (FMU) between December 2011 and February 2019. The schedule of the BR regimen was 90 mg/m^2 × 2 days of bendamustine and 375 mg/m^2 of rituximab every 28 days. We excluded a patient with many missing values. Thus, 60 patients who were followed up for 2 years after the end of BR treatment were included in the present study. This study was approved by the local ethics committee of Aizu Medical Center of FMU, and carried out in accordance with the relevant guidelines and regulations.

Treatment response and toxicity criteria

Efficacy assessments were performed according to the international consensus on the revised response criteria for malignant lymphoma.[13] According to these criteria, we defined therapy responses as follows: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Physical examination and laboratory tests were used to evaluate adverse reactions and toxicities. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0.

Statistical analysis

All statistical analyses were performed with EZR version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of the R commander designed to add statistical functions frequently used in biostatistics.[14] The Kolmogorov-Smirnov test was used to analyze the normality of the distribution of the parameters.[15] All variables with a normal distribution are expressed as the mean ± standard deviation, and those with a log-normal distribution are expressed as the median with interquartile range (IQR).[16] Overall survival (OS) was defined as the time from the start of BR therapy to death or the date of the last follow-up. Progression-free survival (PFS) was defined as the time from the start of BR therapy to relapse, death, or the date of the last follow-up, whichever occurred first.[17] The OS and PFS rates were estimated according to the Kaplan-Meier method and compared by the log-rank test.[18] The hazard ratios (HRs) and their associated 95% confidence intervals (CIs) for potential prognostic factors were calculated using the Cox proportional hazards regression model. Variables with $p < 0.15$ in the univariate analysis were used as independent variables in the multivariate analysis. Dichotomous variables were compared using Fisher's exact test. We determined the cutoff values of serum IgG and lymphocyte count for the diagnosis of infections by receiver operating characteristic (ROC) curve analy-sis. We then divided the patients according to the cutoff values. Furthermore, we performed multivariate logistic regression analysis to evaluate these values for the detection of infections. All statistical tests were two-sided and a significance level of 0.05 was used.

RESULTS

Patient characteristics

The median age of the 60 patients who received BR therapy for indolent NHL in this study was 69 years (range, 40 to 85 years), and 48.3% of patients were women (Table 1). Eighteen patients had a history of pretreatment regimens. Most patients achieved CR (86.9%) with BR therapy. The proportion of patients with poor Eastern Cooperative Oncology Group performance status (PS) scores (greater than 2) was 11.7%, and the proportion of patients with lactate dehydrogenase (LDH) levels greater than the upper limit of normal was 16.7%. At the time of diagnosis, the proportion of patients with advanced stage (stage III and IV) disease was 68.4%. The lymphoma histology of the patients included 44 FL, 6 MCL, 6 MZL, and 4 MALT lymphoma. In addition, the proportion of patients with skin reactions to BR therapy was 46.7%. The number of infections was significantly higher in the group of patients with rash ($P < 0.001$). There were no significant differences in serum IgG values and lymphocyte counts, the proportions of patients treated with acyclovir, antifungal drugs, and trimethoprim-sulfamethoxazole, or the proportion of patients who received rituximab maintenance between patients receiving BR therapy who had infections and those who did not.

Infections during and after BR therapy were significantly associated with PFS

We focused on the adverse effects of BR treatment, especially infections. Throughout the observational period, 24 patients developed infection. Of the 24 patients with infections, 10 had an infection during the BR therapy period and 14 had an infection during the follow-up period after BR therapy. Infections were mainly bacterial, such as bacterial pneumonia, maxillary sinusitis, gingivitis, and enteritis, during BR therapy. By definition, there were 5 patients with febrile neutropenia of unknown origin. On the other hand, viral infections, such as varicella zoster, hepatitis B virus (HBV) reactivation, and cytomegalovirus (CMV) reactivation, were observed after the end of BR therapy (Table 2).

Next, we investigated the influence of infections on the prognosis of patients who received BR therapy. We performed univariate analyses for OS and PFS with log-rank tests (Table 3). OS was not significantly influenced by infections ($P = 0.33$). However, PFS was significantly influenced by the pretreatment regimen ≥1 ($P = 0.013$). We extracted factors with a p-value less than 0.15 for multivariate analyses, including age ≥75 ($P = 0.051$) and PS ≥2 ($P = 0.096$) for OS, and infections ($P = 0.014$) for PFS.

Furthermore, we performed multivariate analysis of PFS

Table 1. Patient characteristics in this study

Variable	Total patients (n=60)	Infection		P-value
		Negative (n=36)	Positive (n=24)	
Age, median (range)	69 (40-85)			
Sex, n (%)				
Female	29 (48.3)	20 (55.6)	9 (37.5)	0.192
Male	31 (51.7)	16 (44.4)	15 (62.5)	
Histology				
FL	44 (73.3)	28 (77.8)	16 (66.7)	0.49
MCL	6 (10)	2 (5.5)	4 (16.7)	
MZL includes MALT lymphoma	10 (16.7)	6 (16.7)	4 (16.7)	
Number of pretreatment regimens				
0	42 (70)	22 (61.1)	20 (83.3)	0.088
≥1	18 (30)	14 (38.9)	4 (16.7)	
Response to bendamustine				
CR	53 (88.3)	34 (94.4)	19 (79.2)	0.12
PR	6 (10)	2 (5.6)	4 (16.7)	
SD	0 (0)	0 (0)	0 (0)	
PD	1 (1.7)	0 (0)	1 (4.1)	
Stages				
I	5 (8.3)	2 (5.6)	3 (12.5)	0.38
II	14 (23.4)	11 (30.5)	3 (12.5)	
III	15 (25)	9 (25)	6 (25.0)	
IV	26 (43.3)	14 (38.9)	12 (50)	
Bone marrow involvement				
Negative	42 (70)	26 (72.2)	16 (66.7)	0.78
Positive	18 (30)	10 (27.8)	8 (33.3)	
ECOG PS, n (%)				
0-1	53 (88.3)	33 (91.7)	20 (83.3)	0.42
2-4	7 (11.7)	3 (8.3)	4 (16.7)	
Skin reaction				
Negative	32 (53.3)	26 (72.2)	6 (25)	< 0.001
Positive	28 (46.7)	10 (27.8)	18 (75)	
LDH				
<240	50 (83.3)	31 (86.1)	19 (79.2)	0.50
≥240	10 (16.7)	5 (13.9)	5 (20.8)	
IgG replacement				
Yes	10 (16.7)	3 (8.3)	7 (29.2)	0.073
No	50 (83.3)	33 (91.7)	17 (70.8)	
Aciclovir				
Yes	38 (63.3)	22 (61.1)	16 (66.7)	0.79
No	22 (36.7)	14 (38.9)	8 (33.3)	
Anti-fungal drugs				
Yes	49 (81.7)	29 (80.6)	20 (83.3)	1.00
No	11 (18.3)	7 (19.4)	4 (16.7)	
trimethoprim-sulfamethoxazole				
Yes	58 (96.7)	34 (94.4)	24 (100)	0.51
No	2 (3.3)	2 (5.6)	0 (0)	
Baseline serum IgG (mg/dL)	1023 (3-2130)	1039 (550-2130)	1023 (3-1587)	0.91
Baseline lymphocyte count (μL)	1256 (500-12136)	1311 (529-8512)	1078 (500-12136)	0.54

Abbreviations: FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; MALT, mucosa associated lymphoid tissue; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ECOG PS, Eastern Cooperative Oncology Group Performance Status; LDH, lactate dehydrogenase.

with the Cox proportional hazards model to control confounding factors. PFS analysis was evaluated by adding age $\geq$75, number of pretreatment regimens $\geq$1, PS $\geq$2, and infections to the independent factors (Table 4). PFS was significantly associated with the number of pretreatment regimens $\geq$1 (HR, 4.43; 95% CI, 1.79–11.0; P = 0.0012) and incidence of infections (HR, 3.45; 95% CI, 1.35–8.8; P = 0.0097).

The onset of infection may be predicted by lymphocytopenia and hypogammaglobulinemia at the first cycle of BR therapy

Hypogammaglobulinemia and lymphocytopenia are representative adverse effects of BR therapy. We hypothesized that observing the transition of these values will enable us to predict the incidence of infections related to BR therapy and investigated these values. Baseline serum IgG concentrations and lymphocyte counts were not significantly different between patients with or without infections (Table 1). In patients with infections during the observation period, the minimum serum IgG values (Fig. 1a) and lymphocyte counts (Fig. 1b) at the first cycle of BR therapy and at the end of BR therapy were significantly lower than those in patients without infections. Hypogammaglobulinemia persisted from BR treatment to 24 months after the end of therapy in patients who developed infections. However, there were no significant differences in lymphocyte counts between patients with and without infections.

Next, we performed ROC analysis to determine cutoff values for IgG values and lymphocyte counts for predicting infections during and after BR therapy (Fig. 2). In the ROC curve analysis, the minimum serum IgG concentration and lymphocyte count at the first cycle of BR therapy for infections throughout the observation period were 806 mg/dL and 100/μL, respectively. In addition, serum IgG values and lymphocyte counts at the end of BR therapy for infections after BR therapy were 536 mg/dL and 242/μL, respectively. Using these results, we set the cutoff values of serum IgG concentration and lymphocyte counts at the first cycle and at the end of BR therapy as 800 mg/dL and 100/μL, and 530 mg/dL and 240/μL, respectively. We then divided the patients into two groups and performed Fisher's exact test. Minimum IgG values and lymphocyte counts at the first cycle of BR therapy were significantly associated with infections throughout the observation period (P = 0.0012 and P < 0.001). There were no significant differences in the proportion of patients who received IgG replacement therapy (Table 5). Moreover, IgG values at the end of BR therapy were slightly associated with infections after BR therapy (P = 0.051). More patients with hypogammaglobulinemia at the end of BR therapy received IgG replacement than those without hypogammaglobulinemia (Table 6).

Lastly, we performed multivariate analyses for infections by logistic regression analysis (Table 7). Based on Fisher's exact test, we included age $\geq$75, skin reaction, minimum IgG values, and minimum lymphocyte counts at the first cycle of BR therapy for infections throughout the observation period, and age $\geq$75, skin reaction, IgG values, and lymphocyte counts at the end of BR therapy for infections after BR treatment. Infections throughout the observation period were significantly associated with a minimum serum IgG value <800 (odds ratio [OR], 5.4; 95% CI, 1.17–24.8; P = 0.03) and minimum lymphocyte counts at the first cycle of BR therapy <100 (OR, 10.4; 95% CI, 1.96–55.6; P = 0.006). Furthermore,

Table 2. The details of infections in patients during and after bendamustine-rituximab therapy

	During therapy	After therapy
Total infection (/60), n (%)	10 (16.7)	14 (23.3)
Bacterial pneumonia	2	5
Febrile neutropenia	5	0
Maxillary sinusitis	0	1
Gingivitis	0	1
Enteritis	1	0
Viral infection	1	5
Varicella zoster	1	3
Hepatitis B virus reactivation	0	1
Cytomegalovirus antigenemia-positive	0	1
Pneumocystis pneumonia	1	0
Fever of unknown origin	0	2

Table 3. Univariate analysis of overall survival and progression-free survival

	Overall survival		Progression-free survival	
Variable	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age >75	3.77 (0.99–14.3)	0.051	1.42 (0.58–3.52)	0.44
Sex (female)	1.95 (0.49–7.82)	0.34	0.99 (0.42–2.28)	0.98
Number of pretreatment regimens $\geq$1	1.68 (0.45–6.27)	0.44	2.84 (1.25–6.47)	0.013
Total cycles of BR therapy $\geq$5	0.99 (0.25–3.81)	0.98	1.08 (0.45–2.62)	0.86
Stage $\geq$III	4.20 (0.52–33.6)	0.18	1.78 (0.65–4.82)	0.26
PS $\geq$2	3.91 (0.79–19.5)	0.096	2.97 (0.99–8.93)	0.052
LDH (>UNL)	1.65 (0.79–3.45)	0.18	1.79 (0.66–4.87)	0.25
Bone marrow involvement	2.26 (0.61–8.44)	0.22	1.34 (0.56–3.18)	0.51
Rash	1.40 (0.37–5.23)	0.62	1.73 (0.74–4.04)	0.21
Infection (entire period)	1.93 (0.52–7.19)	0.33	1.85 (0.81–4.22)	0.14

Abbreviations: BR, bendamustine-rituximab therapy; PS, performance status; LDH, lactate dehydrogenase; UNL, upper normal limit.

**Table 4.** Multivariate analysis of progression-free survival using the Cox proportional hazards model

Variable	Hazard ratio (95% CI)	P-value
Progression-free survival		
Age ≥75	1.80 (0.59–5.5)	0.30
Number of pretreatment regimens ≥1	4.43 (1.79–11.0)	0.0012
PS ≥2	1.69 (0.44–6.4)	0.44
Infection	3.45 (1.35–8.8)	0.0097

Abbreviation: PS, performance status.

infections after BR therapy were significantly associated with skin reactions (OR, 4.65; 95% CI, 1.13–19.1; $P = 0.033$).

DISCUSSION

Based on the present study, the PFS of patients who receive BR treatment is significantly influenced by the incidence of infections. Minimum IgG values and lymphocyte counts at the first cycle of BR therapy were significantly associated with the incidence of infections during and after BR therapy. In addition, serum IgG values at the end of BR therapy were significantly associated with the incidence of infections after BR treatment. This suggested that the risk

a

b
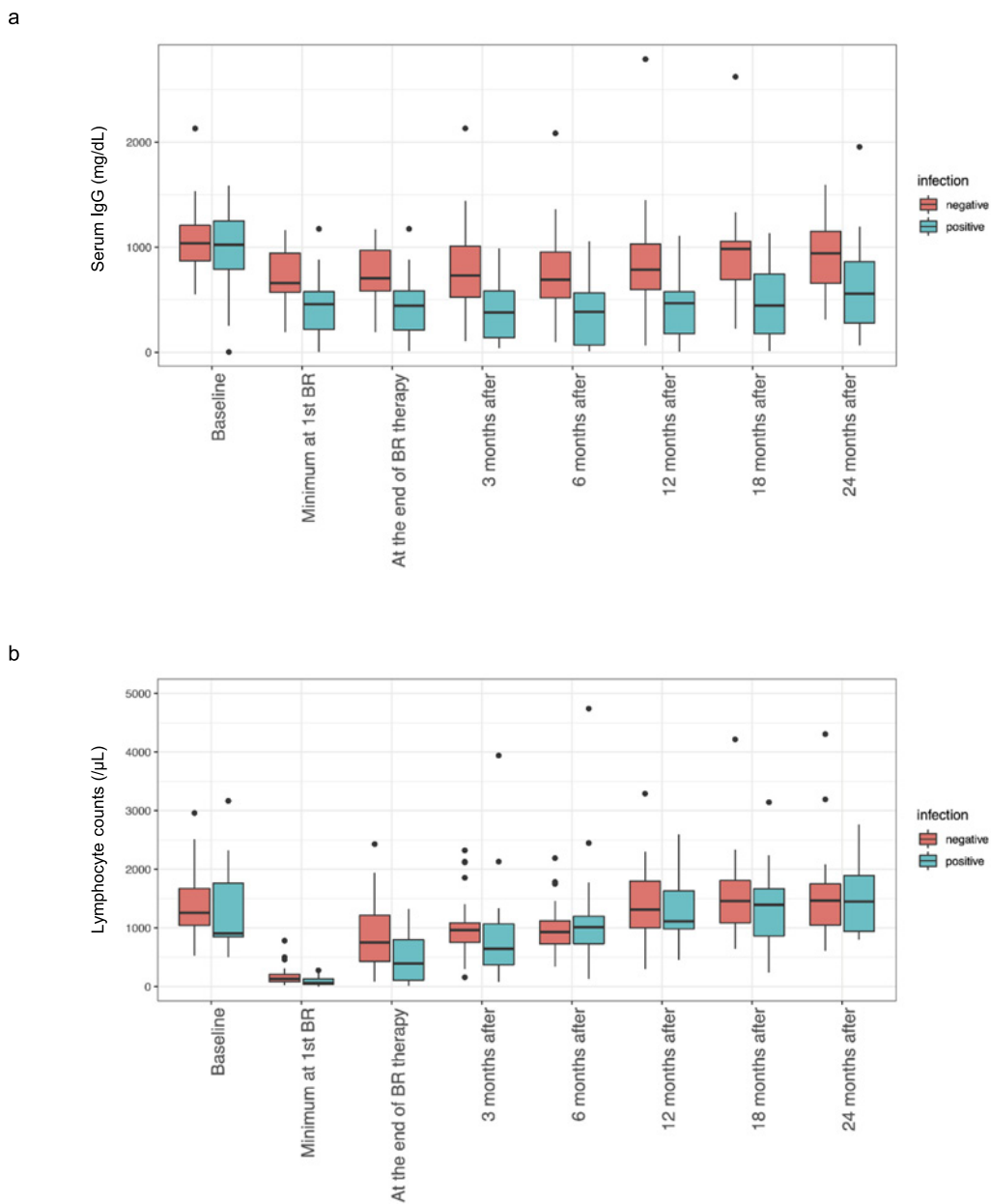
Fig. 1. Transition of serum IgG and lymphocyte counts
(***a***) Serum IgG concentration and (***b***) lymphocyte counts during BR therapy and after BR therapy are shown. P-values were calculated by the Student's t test.

a

b

c

d
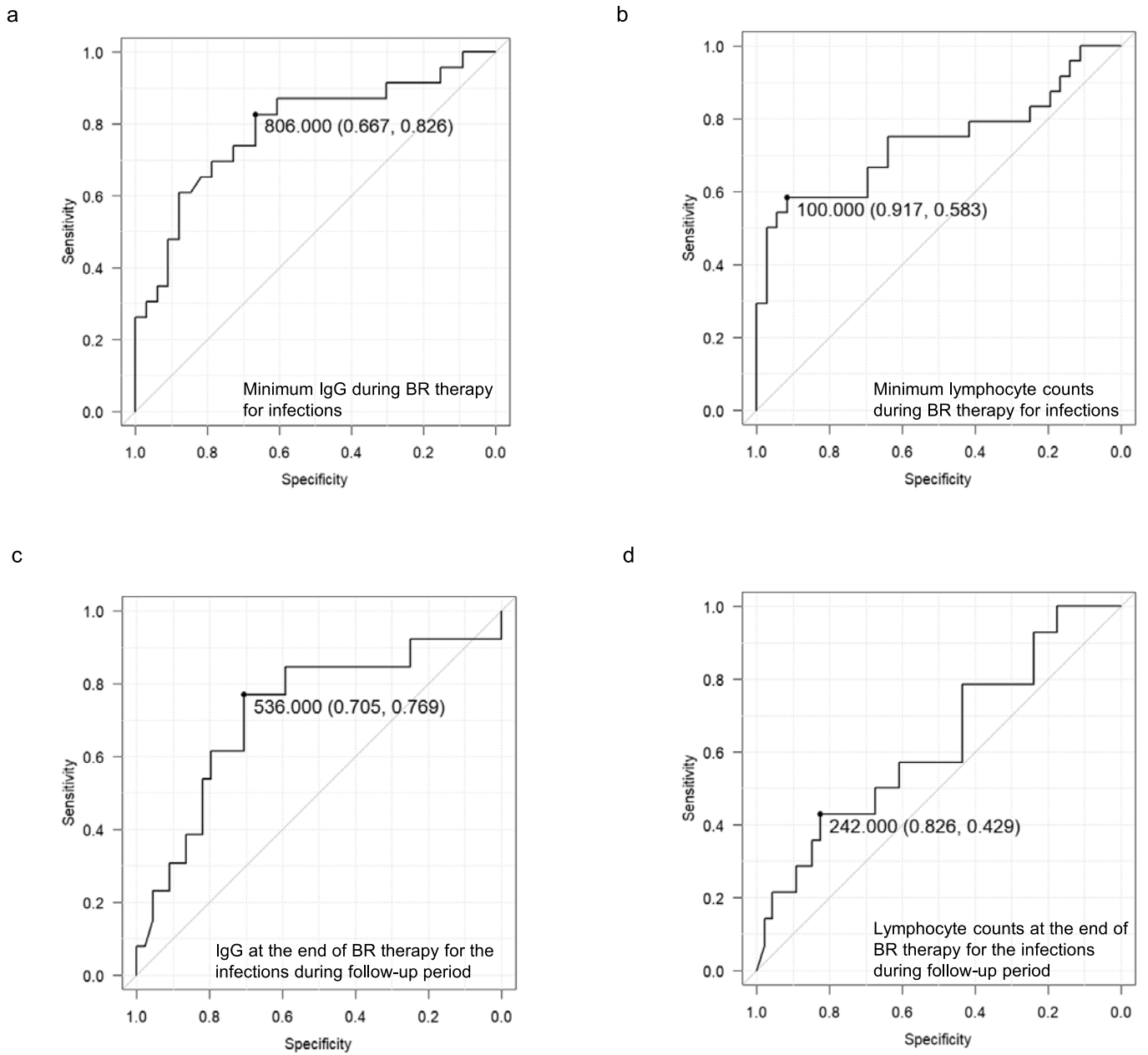
Fig. 2. ROC curve analysis for infections
(*a*) ROC curves of minimum IgG concentration and (*b*) minimum lymphocyte counts for infections throughout the period are shown. (*c*) ROC curves of serum IgG value and (*d*) lymphocyte counts at the end of BR therapy for infections after BR therapy are shown.

Table 5. Fisher's exact test for infection throughout the period

	IgG levels		P-value	Lymphocyte counts		P-value
	< 800	≥ 800		< 100	≥ 100	
Infections throughout the period	N=56			N=60		
Positive	18 (78.3)	5 (21.7)	0.0012	5 (35.7)	9 (64.3)	<0.001
Negative	11 (33.3)	22 (66.7)		8 (17.4)	38 (82.6)	
IgG replacement	N=57			N=60		
Yes	7 (77.8)	2 (22.2)	0.15	4 (40)	6 (60)	0.74
No	22 (46.8)	25 (53.2)		24 (48)	26 (52)	

Table 6. Fisher's exact test for infection after BR therapy

	IgG levels		P-value	Lymphocyte counts		P-value
	< 530	≥ 530		< 240	≥ 240	
Infection after BR therapy	N=57			N=60		
Positive	8 (61.5)	5 (38.5)	0.051	5 (35.7)	9 (64.3)	0.16
Negative	13 (29.5)	31 (70.5)		8 (17.4)	38 (82.6)	
IgG replacement	N=57			N=60		
Yes	7 (70)	3 (30)	0.028	2 (20)	8 (80)	1
No	14 (29.8)	33 (70.2)		11 (22)	39 (78)	

Abbreviation: BR, bendamustine-rituximab therapy.

Table 7. Multivariate analysis for infection by logistic regression analysis

Variable	Odds ratio (95% CI)	P-value
Infection throughout the period		
Age ≥75	1.27 (0.25–6.41)	0.77
Skin reaction	3.54 (0.77–16.3)	0.09
Minimum IgG at 1st BR <800	5.4 (1.17–24.8)	0.03
Minimum lymphocyte count at 1st BR <100	10.4 (1.96–55.6)	0.006
Infection after BR therapy		
Age ≥75	0.88 (0.20–3.88)	0.86
Skin reaction	4.65 (1.13–19.1)	0.033
IgG at end of BR therapy <530	1.98 (0.53–7.39)	0.31
Lymphocyte count at the end of BR therapy <240	2.77 (0.67–11.5)	0.16

Abbreviation: BR, bendamustine-rituximab therapy.

for infections with BR treatment is predictable using minimum serum IgG values and lymphocyte counts at the first cycle. The risk for infections related to BR therapy in the follow-up period may also be evaluated by prolonged hypogammaglobulinemia.

A previous clinical study reported hypogammaglobulinemia (IgG < 500 mg/dL) at the end of BR therapy in 20–30% of patients.[2] In our study, lower serum IgG values (IgG < 536 mg/dL) at the end of BR therapy were observed in 38.6% of patients (22/57). Although our patient group was older than those in previous studies, the proportion of patients with hypogammaglobulinemia was not different.

Our study suggested that low minimum serum IgG values (< 806/μL) and low minimum lymphocyte counts (< 100/μL) at the first BR therapy cycle are predictors for the incidence of infections during and after BR treatment. Physicians may consider whether to continue BR therapy using these biomarkers.

In addition, serum IgG values at the end of BR therapy were useful for predicting the incidence of infections after BR therapy. Knowing these values may help in the management of patients during the follow-up period. The serum IgG values of patients with infections were significantly lower than those of patients without infections at 24 months from the end of BR therapy. Moreover, patients with sustained hypogammaglobulinemia at the end of BR therapy required IgG replacement. IgG replacement may have played a role in the reduction of infections, which is why skin reaction was significantly associated with infections after BR

therapy in multivariate analysis. Taken together, our study revealed that patients with hypogammaglobulinemia at the end of BR therapy should be carefully followed up for the incidence of infections and IgG replacement may be considered.

Skin reaction, which often develops approximately 10 days after the start of BR treatment, has been reported to be a unique adverse effect of bendamustine.[7,8,19-21] In patients with skin reactions to bendamustine treatment, the CD8+ T cell number and CD8+:CD4+ cell ratio are increased at the end of treatment.[10] Bendamustine was also reported to markedly suppress CD4+ T cells.[8,22,23] Skin reactions are associated with a decreased number of CD4+ T cells.[10,24-26] Our study suggested that patients with skin reactions caused by bendamustine had an immunosuppressive status with lymphopenia. Patients who develop skin reactions due to BR therapy need to be carefully followed up because they are in an immunosuppressive state.

There are several limitations in this study. First, as the study population included indolent NHL patients, the observation period was insufficient to evaluate OS and PFS. If the observation period was extended, other prognostic factors related to BR treatment may be extracted. In addition, previous reports suggested that BR treatment affects the T cell subset, resulting in skin reactions and infections. However, in our study, this information about T cell subsets was missed. To solve these limitations, a prospective study is warranted.

In conclusion, minimum serum IgG values and lympho-

cyte counts at the first cycle of BR treatment may be predictive markers for infections related to BR treatment. Furthermore, patients with hypogammaglobulinemia at the end of BR therapy need to be followed carefully for infections. To confirm these findings, a prospective study should be performed.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest (COIs).

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