Clinical significance of oxidative stress for untreated patients with diffuse large B-cell lymphoma

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Abstract. Oxidative stress serves an important role in carcinogenesis. The present study investigated the clinical significance of oxidative stress as a prognostic factor for diffuse large B-cell lymphoma (DLBCL). The participants comprised 55 consecutive patients with DLBCL. A commercially available derivatives of reactive oxygen metabolites (d-ROMs) test kit was used to assess oxidant levels. Similarly, a commercially available biological antioxidant potential (BAP) test was used to assess antioxidant levels. The antioxidative/oxidative stress ratio was calculated as d-ROMs/BAP. The median serum concentration of d-ROMs was 425 μM. The levels of d-ROMs were significantly higher in patients with DLBCL than in healthy volunteers (P<0.01). The complete remission (CR) rates in patients with d-ROMs <425 and ≥425 μM were 81.5 and 85.7%, respectively [not significant (NS)]. The 3-year overall survival (OS) rates for patients with d-ROMs <425 and ≥425 μM were 67.2 and 72.0%, respectively (NS). The median BAP was 2,002 μM. The CR rates of patients with BAP <2,002 and ≥2,002 μM were 77.8 and 88.9%, respectively (NS). The 3-year OS rates of patients with BAP <2,002 and ≥2,002 μM were 60.9 and 75.9%, respectively (NS). No significant difference in the d-ROMs/BAP ratio was observed between groups. Multivariate analysis revealed that d-ROMs were an independent prognostic factor for progression-free survival.

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Introduction

Diffuse large B-cell lymphoma (DLBCL) constitutes 25-30% of adult non-Hodgkin lymphomas in developed countries, with higher percentages in developing countries. This pathology is more common among elderly individuals (1). Many investigators have investigated prognostic factors for DLBCL. We have previously reported various prognostic factors for DLBCL (2-13). Today, the most reliable and established prognostic factors for DLBCL are the International Prognostic Index (IPI) and the revised IPI (R-IPI) (14,15). Usually, the pathogenesis of cancer cells is considered to involve high levels of reactive oxygen species (ROS) because of metabolic and signaling abnormalities. ROS are believed to promote cancer progression through the activation of oncogenic signaling pathways and damage to DNA (16). Oxidative stress can be defined as an imbalance between the pro- and anti-oxidant responses of the cell. Oxidative stress may also result from overproduction of ROS or insufficient neutralization of ROS by anti-oxidants (17).

The measured concentration is considered to be directly proportional to the quantity of reactive oxygen metabolites (ROMs) affected by active ROS and free radicals. Measuring ROMs thus enables quantitative evaluation of the condition of oxidative stress throughout the human body (18). Quantification of derivatives of ROMs (d-ROMs) is a simple method for detecting hydroperoxide levels (19), and clinical trials have shown that the d-ROMs test is useful for evaluating oxidative stress (19,20). Biological antioxidant potential (BAP) can be measured simultaneously.

ROS function may be a key to many impaired biological processes, including cancers. Various investigators have reported that oxidative stress plays an important role in carcinogenesis, including for lung cancer (21-23), hepatocellular carcinoma (24,25), colorectal cancer (26), and ovarian cancer (27). However, we could only identify one report that investigated associations between oxidative stress and hematological malignancies (28). Here, we aimed to

investigate the role of oxidative stress as a prognostic factor for DLBCL in a retrospective analysis.

Patients and methods

Study design. This retrospective study was organized by Gifu University Graduate School of Medicine (Gifu, Japan). Participants were patients with untreated CD20-positive DLBCL at Gifu University Hospital. The initial cohort comprised 55 consecutive patients histologically diagnosed with DLBCL between December 2012 and March 2016 according to the 2008 classification of the World Health Organization (WHO) (29). All follow-up data were updated as of April 12, 2019. Thirty-six healthy volunteers (10 men, 26 women) served as a control group. All patients provided written informed consent to participate in the study according to the guidelines of our institution and the Declaration of Helsinki. Samples were acquired during routine diagnostic assessments. This study was approved by the institutional review board at our institution (Gifu University Graduate School of Medicine, approval no. 2018-003).

Oxidative stress and other determinations. Oxidative stress was investigated by measuring serum hydroperoxide concentrations according to the d-ROMs test (Diacron International srl) using a free radical elective evaluator, FREE (Diacron International srl), as described previously (19,20,24,30). Similarly, a commercially available BAP test was used to assess antioxidant levels (Diacron International srl), as described previously (31). The antioxidative/oxidative stress ratio was calculated as BAP/d-ROMs. Peripheral white blood cell count (WBC) and serum concentrations of lactate dehydrogenase (LDH), soluble interleukin 2 receptor (sIL-2R), and C-reactive protein (CRP) were determined on admission.

Treatment strategy. Patients received 6-8 cycles of either R-CHOP or R-THP-COP. These regimens comprised rituximab (R; 375 mg/m^2, as a 4-h intravenous (i.v.) drip infusion on day 1), cyclophosphamide (C; 750 mg/m^2, as a 2-h i.v. drip infusion on day 3), doxorubicin (H; 50 mg/m^2, as a 30-min i.v. drip infusion on day 3) or tetrahydropyranyl-adriamycin (THP; 50 mg/m^2, as a 30-min i.v. drip infusion on day 3), vincristine (O; 1.4 mg/m^2, maximal dose 2.0 mg i.v. as a bolus over 5 min on day 3), and prednisolone [P; 100 mg/day per os (p.o.) on days 3-7]. The R-THP-COP regimen included THP, an anthracycline derivative of doxorubicin reportedly offering lower cardiotoxicity than doxorubicin (32,33). Our previous prospective randomized study found no significant differences in remission or survival rates between CHOP and THP-COP therapies (34). In addition, we reported the utility and safety of R-THP-COP from a single-arm phase II study (35,36) and a randomized phase III study (37). Granulocyte colony-stimulating factor (G-CSF) was administered at the discretion of the physician. Patients with a bulky mass received radiotherapy after chemotherapy. Patients who relapsed or in whom disease progressed after R-CHOP or R-THP-COP, and those who were resistant to R-CHOP or R-THP-COP underwent salvage chemotherapy with R-P-IMVP-16/CBDCA (rituximab, methylprednisolone, ifosfamide, methotrexate, etoposide, and carboplatin) (38,39). A proportion of patients with refractory or relapsed DLBCL who responded to R-P-IMVP-16/CBDCA received high-dose chemotherapy followed by autologous peripheral blood stem cell transplantation.

Response criteria. Treatment response was evaluated after the 2nd, 4th, 6th, and final cycles of chemotherapy. Treatment responses were categorized after repeated physical examinations, radiological studies, gallium scintigraphy, fluorodeoxyglucose-positron emission tomography, and bone-marrow evaluation according to the 2007 International Working Group Criteria (40).

Statistical analysis. Data are expressed as median. Differences in mean values were tested using the nonparametric Mann-Whitney U-test and Kruskal-Wallis test. For comparisons among ≥3 groups, differences in mean values were tested using the nonparametric Kruskal-Wallis test followed by Dunn's post hoc test. Spearman's correlation coefficient was used to test correlations between d-ROMs and other serum markers. Effects of d-ROMs and other serum markers of survival were examined by univariate analyses using the log-rank test based on Kaplan and Meier methods (41). Multivariate analysis was performed using the Cox proportional-hazards regression technique to define the prognostic significance of selected variables including d-ROM and BAP. Values of $P<0.05$ were considered significant.

Results

Patient characteristics. A total of 55 patients were enrolled in the present study. Table I summarizes the clinical characteristics of patients (median age, 72 years; range, 36-93 years). Thirty-six healthy volunteers were enrolled (median age, 50.5 years; range, 25-82 years) (Table I). A significant difference in age was identified between DLBCL patients and controls ($P<0.01$).

d-ROMs and BAP in DLBCL patients and healthy controls. Median d-ROMs concentration was significantly increased among healthy volunteers (329 μM) compared to DLBCL patients (425 μM; $P<0.001$) (Fig. 1A). In contrast, median BAP values were significantly decreased in DLBCL patients (2,002 μM) compared to healthy volunteers (2,352 μM; $P<0.001$) (Fig. 1B). In addition, the d-ROMs/BAP ratio was significantly higher in DLBCL patients (0.203) than in healthy volunteers (0.137; $P<0.001$) (Fig. 1C). Cut-offs were 425 μM for d-ROMs, 2,002 μM for BAP, and 0.203 for d-ROM/BAP, all of which essentially represented median values for all DLBCL patients.

Correlations between d-ROMs and other markers in DLBCL patients. Table II shows correlations between d-ROMs, BAP, d-ROMs/BAP and other markers in DLBCL patients. No significant correlation existed between d-ROMs and clinical stage in DLBCL patients. Significant correlations existed between d-ROMs and LDH ($P<0.01$), between d-ROMs and sIL-2R ($P<0.001$), between d-ROMs and IPI ($P<0.05$), between d-ROMs and B symptoms ($P<0.001$), and between d-ROMs and bulky disease ($P<0.01$) in patients with DLBCL. Significant correlations existed between BAP and IPI ($P<0.001$). No significant correlations existed between BAP and clinical

Table I. Clinical characteristics of the patients with diffuse large B cell lymphoma (n=55).

Variable	No. (%)
Sex	
Male	36 (65.0)
Female	19 (35.0)
Age, years	
<61	7 (12.7)
≥61	48 (87.3)
PS	
0, 1	46 (84.0)
2-4	9 (16.0)
LDH	
Normal	19 (35.00
Increased	36 (65.0)
Extranodal sites	
0, 1	37 (67.3)
≥2	18 (32.7)
Clinical stage	
I/II	17 (31.0)
III/IV	38 (69.0)
B symptom	
Absence	13 (24.0)
Presence	42 (76.0)
Bulky disease	
Absence	47 (85.0)
Presence	8 (15.0)
sIL-2R, U/ml	
<2,000	38 (69.0)
≥2,000	17 (31.0)
IPI	
Low	11 (20.0)
Low-intermediate	13 (24.0)
High-intermediate	21 (38.0)
High	10 (18.0)
R-IPI	
Very good	1 (1.8)
Good	24 (43.6)
Poor	30 (54.50

OS, overall survival; PFS, progression-free survival; d-ROM, derivatives of reactive oxygen; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; IPI, international prognostic index; R-IPI, revised IPI.

stage, between BAP and sIL-2R, between BAP and B symptoms, between BAP and LDH, between BAP and performance status (PS) or between BAP and bulky disease in patients with DLBCL. Significant correlations existed between d-ROM/BAP and clinical stage (P<0.05), between d-ROM/BAP and sIL-2R (P<0.0001), between d-ROM/BAP and IPI (P<0.05), and between d-ROM/BAP and B symptoms (P<0.001) in patients with DLBCL.

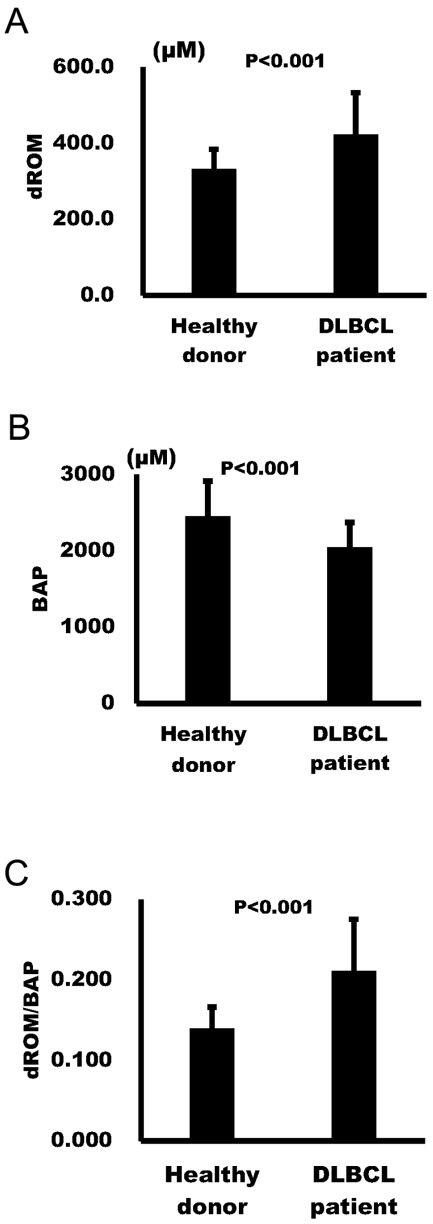

Figure 1. Levels of d-ROMs, BAP and d-ROMs/BAP ratio in patients with DLBCL and healthy controls. (A) Median concentration of d-ROMs was significantly increased in patients with DLBCL (425 μM) compared with healthy volunteers (329 μM; P<0.001). (B) Median BAP concentration was significantly decreased in patients with DLBCL (2,002 μM) compared with healthy volunteers (2,352 μM; P<0.001). (C) Median d-ROM/BAP ratio was significantly higher in patients with DLBCL (0.203) than in healthy volunteers (0.137; P<0.001). Error bars indicate the standard deviation. d-ROMs, derivatives of reactive oxygen metabolites; BAP, biological antioxidant potential; DLBCL, diffuse large B-cell lymphoma.

Analysis of response to therapy. Table III shows the results of analysis of complete remission (CR) rates in DLBCL patients. The CR rate for all DLBCL patients was 83.6%. CR rates of patients with d-ROM <425 and ≥425 μM were 81.5 and 85.7%, respectively [not significant (NS)]. CR rates of patients with BAP <2,002 and ≥2,002 μM were 77.8 and 88.9%, respectively (NS). CR rates of patients with d-ROM/BAP <0.203 and ≥0.203 were 88.9 and 77.8%,

Table II. Association between oxidative stress markers and other markers in diffuse large B cell lymphoma.

	d-ROM (μM)			BAP (μM)			d-ROM/BAP		
Variable	Median	Range	P-value	Median	Range	P-value	Median	Range	P-value
Sex									
Male	426	262-660	0.3427	1999	1468-2653	0.8527	0.20	0.124-0.432	0.7028
Female	381	171-658		2126	1571-2812		0.21	0.100-0.360	
Age, years									
<61	415	303-660	0.6483	2213	1640-2609	0.2450	0.23	0.129-0.253	0.6483
≥61	426	171-658		1998	1468-2812		0.20	0.100-0.432	
PS									
0, 1	402	171-660	0.3011	2129	1538-2812	0.0248	0.19	0.100-0.360	0.0248
2-4	497	280-634		1728	1468-2457		0.29	0.181-0.432	
LDH									
Normal	364	247-474	0.0372	2175	1626-2563	0.4515	0.17	0.100-0.266	0.0491
Increased	440	171-660		1981	1484-2812		0.23	0.101-0.432	
Extranodal sites									
0, 1	435	171-660	0.6307	1998	1468-2609	0.2911	0.19	0.101-0.432	0.5036
≥2	434	247-622		2113	2113-2812		0.21	0.100-0.297	
Clinical stage									
I/II	378	269-474	0.0618	2178	1571-2572	0.0900	0.17	0.124-0.245	0.0469
III/IV	435	171-660		1981	1468-2812		0.22	0.100-0.432	
B symptom									
Absence	378	171-660	0.0054	2138	1538-2653	0.3043	0.19	0.100-0.293	0.0021
Presence	553	321-658		1908	1468-2812		0.29	0.165-0.432	
Bulky disease									
Absence	389	171-658	0.0089	2059	1538-2812	0.4118	0.19	0.100-0.360	0.0018
Presence	552	381-660		1736	1468-2609		0.26	0.242-0.432	
sIL-2R, U/ml									
<2,000	378	247-660	0.0112	2168	1538-2812	0.0121	0.18	0.100-0.266	0.0066
≥2,000	507	171-658		1825	1468-2543		0.28	0.101-0.432	
IPI									
Low	364	292-474	0.0394	2203	1782-2563	0.0023	0.17	0.129-0.228	0.0122
Low-intermediate	389	269-660		2126	1697-2609		0.19	0.124-0.266	
High-intermediate	415	171-658		1773	1468-2482		0.24	0.1-0.432	
High	521	343-622		2195	1825-2812		0.22	0.129-0.297	
R-IPI									
Very good (0 points)[a]	378	378	0.1507	2213	2213	0.0956	0.17	0.171	0.0470
Good (1 and 2 points)	376	269-660		2176	1697-2609		0.18	0.124-0.266	
Poor (3-5 points)	442	171-658		1904	1468-2812		0.23	0.10-0.432	

[a]The Very good group included only 1 patient. d-ROM, derivatives of reactive oxygen metabolities; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; IPI, international prognostic index; R-IPI, revised IPI.

respectively (NS). No factors were significantly associated with CR rates in DLBCL patients.

Oxidative stress as a prognostic factor in DLBCL. Table IV shows the results of univariate analyses for survival rates in DLBCL. Median follow-up period was 26.2 months. Three-year overall survival (OS) rates for patients with d-ROMs <425 and ≥425 μM were 67.2 and 72.0%, respectively (NS, Fig. 2A). Three-year OS rates for patients with BAP <2,002 and ≥2,002 μM were 60.9 and 75.9%, respectively (NS, Fig. 2B). Three-year OS rates for patients with d-ROMs/BAP <0.203 and ≥0.203 were 65.5 and 71.6%, respectively (NS, Fig. 2C). Other factors associated with significantly worse OS were poor PS (>1) and unfavorable IPI (high intermediate and high risk groups). Three-year progression-free survival (PFS) rates for patients with d-ROMs <425 and ≥425 μM were 66.7 and 65.1%, respectively (NS, Fig. 2D). Three-year PFS rates for patients with BAP <2,002 and ≥2,002 μM were 54.0 and 73.7%, respectively (NS, Fig. 2E). Three-year PFS rates for patients with d-ROMs/BAP <0.203 and ≥0.203 were 66.7 and 65.1%, respectively (NS, Fig. 2F). Other factors associated with significantly worsened PFS were advanced stage (III or IV), and unfavorable IPI (HI and H risk groups) (Table I).

Multivariate analyses for OS and PFS (Table V). Multivariate analyses identified age, PS, clinical stage, and sIL-2R as

Table III. Univariate analysis of remission rate in diffuse large B cell lymphoma.

Variable	Total no. of patients	CR	
		%	P-value
All patients	55	83.6	
Sex			
Male	36	78.9	0.4947
Female	19	86.1	
Age, years			
<61	7	85.7	0.8736
≥61	48	83.3	
PS			
0, 1	46	84.8	0.6034
2-4	9	77.8	
LDH			
Normal	19	94.7	0.106
Increased	36	77.8	
Extranodal sites			
0, 1	37	86.5	0.9662
≥2	18	77.8	
Clinical stage			
I/II	17	88.2	0.6198
III/IV	38	81.6	
B symptom			
Absence	13	81.0	0.3335
Presence	42	92.3	
Bulky disease			
Absence	47	87.2	0.0804
Presence	8	62.5	
sIL-2R, U/ml			
<2,000	38	81.6	0.5375
≥2,000	17	88.2	
IPI			
Low	11	100.0	0.0577
Low-intermediate	13	92.3	
High-intermediate	21	66.7	
High	10	90.0	
R-IPI			
Very good	1	100.0	0.0982
Good	24	91.7	
Poor	30	76.7	
d-ROM			
<425 μM	22	81.5	0.6714
≥425 μM	24	85.7	
BAP			
≥2,002 μM	24	88.9	0.2488
<2,002 μM	21	77.8	
d-ROM/BAP			
<0.203	24	88.9	0.2488
≥0.203	21	77.8	

CR, complete remission; d-ROM, derivatives of reactive oxygen metabolities; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; IPI, international prognostic index; R-IPI, revised IPI.

Table IV. Univariate analysis of survival rate in diffuse large B cell lymphoma.

	3 year-OS			3 year-PFS		
Variable	No. of patients	%	P-value	No. of patients	%	P-value
All patients	55	69.3		55	65.5	
Age, years						
<61	7	100.0	0.1129	7	71.4	0.5840
≥61	48	58.1		48	47.8	
PS						
0, 1	46	76.1	0.0101	46	71.3	0.0940
2-4	9	NR		9	NR	
LDH						
Normal	19	86.1	0.0541	19	89.1	0.0311
Increased	36	50.2		36	45.0	
Extranodal sites						
0, 1	37	72.0	0.8239	37	70.8	0.4569
≥2	18	63.8		18	53.9	
Clinical stage						
I/II	17	87.8	0.3399	17	87.8	0.0433
III/IV	38	61.6		38	55.7	
B symptom						
Absence	13	64.9	0.2503	13	66.1	0.3018
Presence	42	61.5		42	48.5	
Bulky disease						
Absence	47	70.1	0.5217	47	71.1	0.1580
Presence	8	37.5		8	25.0	
sIL-2R, U/ml						
<2,000	38	70.6	0.8837	38	70.6	0.7164
≥2,000	17	66.5		17	54.5	
IPI						
Low	11	100.0	0.0382	11	100.0	0.0232
Low-intermediate	13	82.1		13	76.9	
High-intermediate	21	52.7		21	49.0	
High	10	54.9		10	43.8	
R-IPI						
Very good	1	NR	0.0533	1	100.0	0.0169
Good	24	85.4		24	82.9	
Poor	30	54.9		30	50.2	
d-ROM						
<425 μM	27	67.2	0.4369	27	66.7	0.4104
≥425 μM	28	72.0		28	65.1	
BAP						
≥2,002 μM	27	75.9	0.2510	27	73.7	0.2055
<2,002 μM	27	60.9		27	54.0	
d-ROM/BAP						
<0.203	27	65.5	0.9217	27	60.2	0.8086
≥0.203	27	71.6		27	70.4	

OS, overall survival; PFS, progression-free survival; d-ROM, derivatives of reactive oxygen metabolities; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; IPI, international prognostic index; R-IPI, revised IPI; NR, not reached.

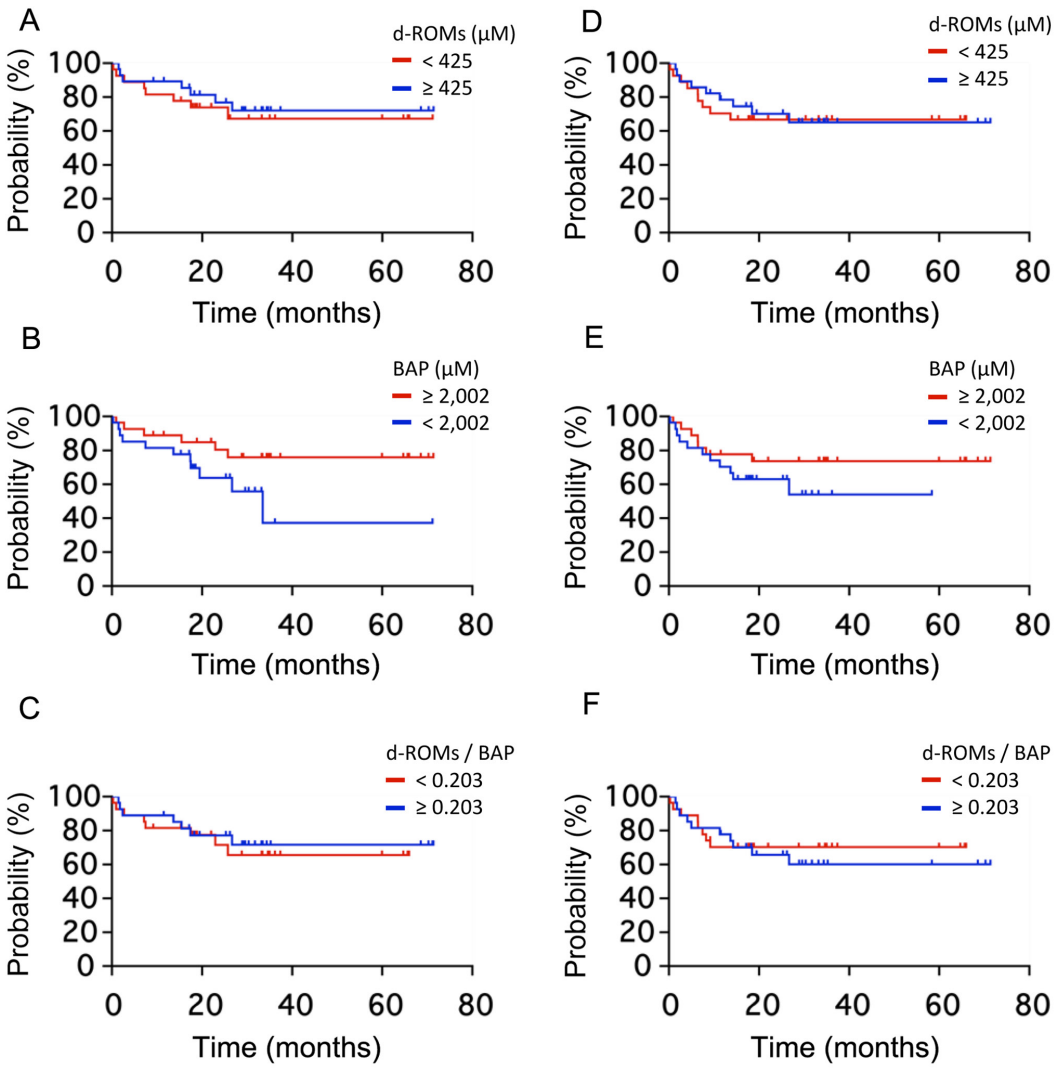

Figure 2. OS and PFS curves according to d-ROMs, BAP and d-ROMs/BAP ratio in patients with DLBCL. (A) 3-year OS rates for patients with d-ROMs <425 and ≥425 μM were 67.2 and 72.0%, respectively (NS). (B) 3-year OS rates for patients with BAP <2,002 and ≥2,002 μM were 60.9 and 75.9%, respectively (NS). (C) 3-year OS rates for patients with d-ROMs/BAP <0.203 and ≥0.203 were 65.5 and 71.6%, respectively (NS). (D) 3-year PFS rates for patients with d-ROMs <425 and ≥425 μM were 66.7 and 65.1%, respectively (NS). (E) 3-year PFS rates for patients with BAP <2,002 and ≥2,002 μM were 54.0 and 73.7%, respectively (NS). (F) 3-year PFS rates for patients with d-ROMs/BAP <0.203 and ≥0.203 were 66.7 and 65.1%, respectively (NS). OS, overall survival; PFS, progression-free survival; d-ROMs, derivatives of reactive oxygen metabolites; BAP, biological antioxidant potential; DLBCL, diffuse large B-cell lymphoma; NS, not significant.

independent prognostic factors for OS, and LDH, clinical stage, and d-ROMs as independent prognostic factors for PFS.

Discussion

Oxidative stress might play an important role in carcinogenesis. Excessive production of ROS has been reported to cause damage to cellular macromolecules such as DNA (42), and can increase levels of various types of DNA damage, including DNA base damage and single- and double-strand breaks (43,44). In addition, excessive production of ROS could interrupt the tumor cell signaling pathways, which are involved in cell growth and survival. This obstruction might lead to cancer promotion and progression. Indeed, many reports have shown that oxidative stress might play important roles in carcinogenesis for some malignancies (21-27). Tsukioka *et al* (21) reported preoperative serum levels of ROMs as a significant independent predictor of nodal involvement in patients with clinical stage I lung adenocarcinoma. Oxidative stress could be reasonably expected to impact the progression of lung cancer, because the lung is the organ most affected by increased oxidative stress. Gencer *et al* (23) reported that serum levels of ROMs were increased in patients with different types of lung cancers and speculated that serum levels of ROMs may offer an index parameter for lung cancer. The role of ROS in colorectal cancer was examined by Inokuma *et al* (26). They reported that serum ROS levels were elevated in proportion to tumor invasion and showed a significant positive correlation with tumor size. Suzuki *et al* (24) reported that hepatocellular carcinoma patients with increased levels of oxidative stress were prone to recurrence after curative treatment.

The present study found a significant difference in age between DLBCL patients and controls. A previous study reported that oxidative stress was associated with mortality in older ages (45). We could not exclude the potential impact of aging on the production of oxidative stressors. Generally,

Table V. Multivariate analysis of OS and PFS in diffuse large B cell lymphoma.

A, OS

Variable	Comparison	Hazard ratio	95% CI	P-value
Age	<61 vs. ≥61 years	3.54	0.40-2.67	0.0082
PS	0,1 vs. 2-4	6.89	1.48-33.33	0.0152
LDH	Normal vs. increased	3.66	0.71-30.13	0.1269
Extranodal sites	0, 1 vs. ≥2	1.76	0.41-7.78	0.4440
Clinical stage	I, II vs. III, IV	9.11	1.42-97.47	0.0189
B symptoms	Absence vs. presence	6.88	0.87-81.02	0.0680
Bulky disease	Absence vs. presence	4.68	0.60-35.32	0.1358
sIL-2R	<2,000 vs. ≥2,000 U/ml	6.01	1.10-47.90	0.0377
d-ROM	<425 vs. ≥425 μM	2.21	0.64-8.22	0.2093
BAP	<2,002 vs. ≥2,002 μM	1.09	0.25-4.62	0.9077

B, PFS

Variable	Comparison	Hazard ratio	95% CI	P-value
Age	<61 vs. ≥61 years	2.59	0.46-22.99	0.3024
PS	0,1 vs. 2-4	2.41	0.61-8.43	0.1974
LDH	Normal vs. increased	9.30	1.78-69.13	0.0069
Extranodal sites	0, 1 vs. ≥2	1.69	0.56-5.12	0.3448
Clinical stage	I, II vs. III, IV	6.73	1.22-56.79	0.0279
B symptoms	Absence vs. presence	1.99	0.49-9.52	0.3435
Bulky disease	Absence vs. presence	3.97	0.87-18.42	0.0747
sIL-2R	<2,000 vs. ≥2,000 U/ml	3.77	0.95-18.10	0.0596
d-ROM	<425 vs. ≥425 μM	3.66	1.09-13.65	0.0361
BAP	<2,002 vs. ≥2,002 μM	2.83	0.87-9.72	0.0837

Multivariate analysis was performed using the Cox proportional-hazards regression technique to define the prognostic significance of selected variables including d-ROM and BAP. OS, overall survival; PFS, progression-free survival; d-ROM, derivatives of reactive oxygen metabolities; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; 95% CI, 95% confidence interval.

elderly individuals comprise the majority of DLBCL patients, and age represents an important prognostic factor for DLBCL. We suspected that the production of oxidative stress might affect not only the carcinogenesis of DLBCL but also the poor prognosis in elderly patients with DLBCL. One possibility is that d-ROMs, BAP, and d-ROMs/BAP ratio might reflect the risk of inflammatory complications such as pneumonia in DLBCL patients. We cannot deny the possibility that inflammatory complications affected d-ROMs and BAP concentrations. When d-ROMs and BAP were measured in DLBCL patients on admission, no cases were complicated with inflammatory diseases such as pneumonia. Indeed, a significant correlation existed between d-ROMs and B symptoms.

In the present study, d-ROMs in DLBCL patients were increased compared to those in healthy volunteers. Similarly, BAP in DLBCL patients was decreased compared to that in healthy volunteers. As a result, the d-ROMs/BAP ratio was significantly higher in DLBCL patients than in healthy volunteers. These results suggest that oxidative stress contributes to carcinogenesis in DLBCL by damaging DNA. Our study could not find any positive correlation between d-ROMs and clinical stage. This result suggests that d-ROMs might not reflect the volume of lymphoma cells directly. Meanwhile, d-ROMs correlated significantly with both sIL-2R and IPI, which are recognized as powerful prognostic factors for DLBCL. Unfortunately, no significant difference in CR rates was seen between patients with d-ROMs <425 and ≥425 μM, BAP <2,002 and ≥2,002 μM, or d-ROMs/BAP <0.203 and ≥0.203. In addition, no significant differences in 3-year OS rates were identified between patients with d-ROMs <425 and ≥425 μM. Similarly, no significant differences were observed for BAP and d-ROMs/BAP ratio. However, multivariate analysis revealed d-ROMs as an independent prognostic factor for DLBCL patients in PFS. This result showed that oxidative stress may impact prognosis in DLBCL patients. At the same time, we should discuss the discrepancy between the results from uni- and multivariate analyses. We considered that some differences in background characteristics exist between high- and low-d-ROMs patients. Such differences in background may have contributed to

discrepancies between results from uni- and multivariate analyses. If we could match backgrounds between high- and low-d-ROMs patients, univariate analyses might reveal some significant differences. Multivariate analysis offers a useful method to address the issue of differences in background characteristics. Indeed, the present study found significant deviations in LDH, B symptoms, and sIL-2R (data not shown). To clarify these problems, prospective studies are required. Recently, Nojima *et al* (28) reported the role of oxidative stress in DLBCL. They measured d-ROMs and BAP in patients with non-Hodgkin's lymphoma (NHL), similar to our study. Defining oxidation stress index (OSI) as OSI=C x (d-ROMs/BAP), where C denotes a coefficient for standardization to set the mean OSI in healthy individuals at 1.0), they reported OSI as significantly higher in DLBCL patients with advanced clinical stage compared to localized stage. They therefore claimed that the OSI might offer a useful clinical marker for NHL. However, they did not show differences in either CR rates or survival rates according to oxidative stress.

The issue of d-ROMs and BAP in lymphoma tissue is very interesting, but unfortunately we did not measure these concentrations in lymphoid tumor tissue. We therefore could not clarify the associations between d-ROMs and BAP concentrations in lymphoma tissue and serum. We consider that d-ROMs and BAP concentrations in lymphoid tumor tissue may correlate with serum concentrations, but serum concentrations of d-ROMs and BAP may also reflect immune responses of the whole body to lymphoid malignancies. Measurement of d-ROMs and BAP concentrations in lymphoid tumor tissue may clarify which cells produce the oxidative stress and the mechanisms by which oxidative stress affects the carcinogenesis of DLBCL.

In the present study, cases with high LDH levels showed higher levels of d-ROMs than those with low LDH levels. This may indicate that LDH reflects global dynamic metabolic reactions, including ROS. In addition, a previous study of population-based cohorts found that levels of d-ROMs were strongly associated with cancer mortality (46). In the present study, concentrations of d-ROMs were significantly higher in DLBCL patients than in healthy volunteers, and oxidative stress may also be associated with an increased risk of DLBCL (47).

In conclusion, levels of d-ROMs were significantly higher in DLBCL patients than in healthy volunteers. Although univariate analysis revealed that oxidative stress did not impact the prognosis of untreated patients with DLBCL, multivariate analysis revealed d-ROMs as an independent prognostic factor for DLBCL patients in PFS. These results showed that oxidative stress plays important roles in carcinogenesis for DLBCL patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HT, MS and TH designed the present study. HN, RM, TM, NN, JK and YK developed the methodology and assessed the authenticity of the raw data. TM, NN, SN, JK, YK, TsT, TM and TaT provided resources. HN, SN, NN, NK, TsT, TM and TaT performed the experiments. HN and TH wrote the original draft. MS and HT reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Investigations were performed in compliance with the principles of good clinical practice outlined in the Declaration of Helsinki and federal guidelines, and had approval by the Medical Review Board of Gifu University Graduate School of Medicine, Gifu, Japan (approval no. 2018-003). Written informed consent was obtained from each participant.

Patient consent for publication

Consent for publication was obtained from any individual person whose data are included in this manuscript.

Competing interests

The authors declare that they have no competing interests.

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