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

HIROSHI NAKAMURA1, TAKESHI HARA1,2, RYOKO MABUCHI1, TAKURO MATSUMOTO1, NOBUHIKO NAKAMURA1, SORANOBU NINOMIYA1, JUNICHI KITAGAWA1, NOBUHIRO KANEMURA1, YUSUKE KITO3, TSUYOSHI TAKAMI3, TATSUHIKO MIYAZAKI4, TAMOTSU TAKEUCHI3, MASAHITO SHIMIZU1 and HISASHI TSURUMI1,2

1First Department of Internal Medicine, Gifu University Graduate School of Medicine, Gifu 501-1194; 2Department of Hematology, Matsunami General Hospital, Kasamatsu-cho, Hashima-gun, Gifu 501-6062; 3Department of Pathology and Translational Research, Gifu University Graduate School of Medicine; 4Department of Pathology, Gifu University Hospital, Gifu 501-1194, Japan

Received May 15, 2020; Accepted July 14, 2021

DOI: 10.3892/mco.2021.2437

Correspondence to: Professor Hisashi Tsurumi, First Department of Internal Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
E-mail: htsuru@gifu-u.ac.jp

Key words: diffuse large B-cell lymphoma, non-Hodgkin's lymphoma, oxidative stress, derivatives of reactive oxygen metabolites test, biological antioxidant potential test

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 77.8 and 88.9%, 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.

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 (Dia cron International srl) using a free radical elective evaluator, FREE (Dia cron International srl), as described previously (19,20,24,30). Similarly, a commercially available BAP test was used to assess antioxidant levels (Dia cron International srl), as described previously (31). The antioxidant/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/m², as a 4-h intravenous (i.v.) drip infusion on day 1), cyclophosphamide (C; 750 mg/m², as a 2-h i.v. drip infusion on day 3), doxorubicin (H; 50 mg/m², as a 30-min i.v. drip infusion on day 3) or tetrahydropyranyl-adriamycin (THP; 50 mg/m², as a 30-min i.v. drip infusion on day 3), vincristine (O; 1.4 mg/m², 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...
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.

**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 (35.0) |
| 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.

**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%,
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

| Variable                              | d-ROM (µM) | BAP (µM) | d-ROM/BAP |
|---------------------------------------|------------|----------|-----------|
| **Median**                            |            |          |           |
| **Range**                             |            |          |           |
| **P-value**                           |            |          |           |
| **Sex**                               |            |          |           |
| Male                                  | 426        | 1999     | 0.20      |
| Female                                | 381        | 2126     | 0.21      |
| **Age, years**                        |            |          |           |
| <61                                   | 415        | 2213     | 0.23      |
| ≥61                                   | 426        | 1998     | 0.20      |
| **PS**                                |            |          |           |
| 0, 1                                  | 402        | 2129     | 0.19      |
| 2-4                                   | 497        | 1728     | 0.29      |
| **LDH**                               |            |          |           |
| Normal                                | 364        | 2175     | 0.17      |
| Increased                             | 440        | 1981     | 0.23      |
| **Extranodal sites**                  |            |          |           |
| 0, 1                                  | 435        | 1998     | 0.19      |
| ≥2                                    | 434        | 2113     | 0.21      |
| **Clinical stage**                    |            |          |           |
| I/II                                  | 378        | 2178     | 0.17      |
| III/IV                                | 435        | 1981     | 0.22      |
| **B symptom**                         |            |          |           |
| Absence                               | 378        | 2138     | 0.19      |
| Presence                              | 553        | 1908     | 0.29      |
| **Bulky disease**                     |            |          |           |
| Absence                               | 389        | 2059     | 0.19      |
| Presence                              | 552        | 1736     | 0.26      |
| **sIL-2R, U/ml**                      |            |          |           |
| <2,000                                | 378        | 2168     | 0.18      |
| ≥2,000                                | 507        | 1825     | 0.28      |
| **IPI**                               |            |          |           |
| Low                                   | 364        | 2203     | 0.17      |
| Low-intermediate                      | 389        | 2126     | 0.19      |
| High-intermediate                     | 415        | 1773     | 0.24      |
| High                                  | 521        | 2195     | 0.22      |
| **R-IPI**                             |            |          |           |
| Very good (0 points)^a                | 378        | 2213     | 0.17      |
| Good (1 and 2 points)                 | 376        | 2213     | 0.17      |
| Poor (3-5 points)                     | 442        | 1904     | 0.23      |

^aThe 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.
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.

| Variable                      | 3 year-OS |          |          | 3 year-PFS |          |          |
|-------------------------------|-----------|----------|----------|------------|----------|----------|
|                               | 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   |
| <2,000 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 metabolites; 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.
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,
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

### 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 metabolites; BAP, biological antioxidant potential; PS, performance status; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin 2 receptor; 95% CI, 95% confidence interval.
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.

Acknowledgements

The authors would like to thank the participating physicians, Dr Yoshikazu Ikoma, Dr Kaneda Yuto, Dr Kimihiro Yamaguechi and Dr Eri Takada. The authors are also grateful to Ms. Chiyoko Sano, Ms. Hitomi Fujisawa, Ms. Miho Yagi and Ms. Eriko Kunishima (all First Department of Internal Medicine, Gifu University Graduate School of Medicine, Gifu, Japan) for their administrative and technical assistance. This abstract was presented at the 17th Japanese Society of Medical Oncology Annual Meeting (July 18-20, 2019 in Kyoto, Japan) and was published as Abstract no. P3-085.

Funding

No funding was received.

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, SN, 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.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, (eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon, pp 291-297, 2017.
2. Yamamoto Y, Goto N, Takemura M, Yamasure W, Yabe K, Takami T, Miyazaki T, Takeuchi T, Shiraki M, Shimizu M, et al: Association between increased serum GP88 (progranulin) concentrations and prognosis in patients with malignant lymphomas. Clin Chim Acta 473: 139-146, 2017.
3. Nakamura N, Hara T, Shibata Y, Matsumoto T, Nakamura H, Ninomiya S, Kito Y, Kitagawa J, Kanemura N, Goto N, et al: Sarcopenia is an independent prognostic factor in male patients with diffuse large B-cell lymphoma. Ann Hematol 94: 2043-2053, 2015.
4. Nakamura N, Goto N, Tsurumi H, Takemura M, Kanemura N, Kasahara S, Hara T, Yasuda I, Shimizu M, Sawada M, et al: Serum level of soluble tumor necrosis factor receptor 2 is associated with the outcome of patients with diffuse large B-cell lymphoma treated with the R-CHOP regimen. Eur J Haematol 91: 322-331, 2013.
5. Goto N, Tsurumi H, Takemura M, Kanemura N, Kasahara S, Hara T, Yasuda I, Shimizu M, Yamada T, Sawada M, et al: Serum soluble CD27 level is associated with outcome in patients with diffuse large B-cell lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone. Leuk Lymphoma 53: 1494-1500, 2012.
6. Goto N, Tsurumi H, Goto H, Shimomura YI, Kasahara S, Hara T, Yasuda I, Shimizu M, Murakami N, Yoshikawa T, et al: Serum soluble interleukin-2 receptor (sIL-2R) level is associated with the outcome of patients with diffuse large B-cell lymphoma treated with R-CHOP regimens. Ann Hematol 91: 705-714, 2012.

7. Ninomiya S, Hara T, Tsurumi H, Goto N, Saito K, Seishima M, Takami T and Moriwaki H: Indoleamine 2,3-dioxygenase expression and serum kynurenine concentrations in patients with diffuse large B-cell lymphoma. Leuk Lymphoma 53: 1143-1145, 2012.

8. Goto N, Tsurumi H, Kasahara S, Kanemura N, Hara T, Yasuda I, Shimizu M, Murakami N, Sawada M, Yamada T, et al: Serum interleukin-18 level is associated with the outcome of patients with diffuse large B-cell lymphoma treated with CHOP or R-CHOP. Eur J Haematol 87: 217-227, 2011.

9. Ninomiya S, Hara T, Tsurumi H, Hoshi M, Kanemura N, Goto N, Kasahara S, Shimizu M, Ito H, Saito K, et al: Indoleamine 2,3-dioxygenase in tumor tissue indicates prognosis in patients with diffuse large B-cell lymphoma treated with R-CHOP. Ann Hematol 90: 409-416, 2011.

10. Yoshikawa T, Hara T, Tsurumi H, Goto N, Hoshi M, Kitagawa J, Kanemura N, Kasahara S, Ito H, Takemura M, et al: Serum concentration of L-kynurenine predicts the clinical outcome of patients with diffuse large B-cell lymphoma treated with R-CHOP. Eur J Haematol 84: 304-309, 2010.

11. Han L, Ninomiya S, Hara T, Goto N, Kanemura N, Yoshikawa T, Kasahara S, Yamada T, Sawada M, Goto H, Fukuno K, et al: Serum soluble Fas level determines clinical outcome of patients with diffuse large B-cell lymphoma treated with CHOP and R-CHOP. J Cancer Res Clin Oncol 135: 1421-1428, 2009.

12. Koijma Y, Tsurumi H, Goto N, Shimizu M, Kasahara S, Yamada T, Sawada M, Ito H, Takemura M, et al: Fas and Fas ligand expression on germinal center type-diffuse large B-cell lymphoma is associated with the clinical outcome. Eur J Haematol 76: 465-472, 2006.

13. Watanuki‑Miyauchi R1, Kojima Y, Tsurumi H, Goto N, Hokuto K, Fukuno K, et al: Survival of patients with diffuse large B-cell lymphoma treated with CHOP and R-CHOP. Cancer Chemother Pharmacol 20: 151‑154, 1987.

14. Sehn LH, Berry B, Chhanabhai M, Fitzgerald C, Gill K, Hoskins P, Klaas R, Savage KJ, Shenker T, Sutherland J, et al: The revised International Prognostic Index (R-IPI) is a better predictor of survival than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 109: 1857‑1861, 2007.

15. Gorrini C, Harris IS and Mak TW: Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12: 93‑108, 2013.

16. Salzmann R, Pácal L, Kaňková K, Tomandl J, Horáková Z, Třováková V, Kodymova K, Lieskovský P, Adamec M, et al: Increased oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12: 395‑406, 2013.

17. Ninomiya S, Hara T, Tsurumi H, Goto N, Saito K, Seishima M, Takami T and Moriwaki H: Indoleamine 2,3-dioxygenase expression and serum kynurenine concentrations in patients with diffuse large B-cell lymphoma. Leuk Lymphoma 53: 1143-1145, 2012.

18.恶心泽义, 塩崎, 他, 等: 单反重组氧化代谢物水平与不同组织病理学类型的类型关系。Cancer Res 73: 520-524, 2006.

19. Suzuki Y, Imai K, Takanaka T, Hanai T, Hayamoto M, Naiki T, Nishizaki Y, Tomita E, Hara T and Moriwaki H: Hepatocellular carcinoma patients with increased oxidative stress levels are prone to recurrence after curative treatment: A prospective case series study using the d-ROM test. J Cancer Res Clin Oncol 139: 845-852, 2013.

20. 池田 Y: 氧化性応力の参加と肝細胞癌の発生。Gastroenterol Sci 41: 1135-1148, 2006.

21. Inokuma T, Haraguchi M, Fujita F, Tajima Y and Kanematsu T: Oxidative stress and tumor progression in colorectal cancer. Hepatogastroenterology 56: 343-347, 2009.

22. 高橋 R, 喜多 K, 東村 R, 藤井 M, 藤原 M, 岩本 J, 久野 G, 郷田 K, B, 藤野 G, 田中 G: 増加された酸化ストレス促進が癌の進行を促進する。Biomed Pharmacother 67: 99-111, 2013.
41. Kaplan EL and Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53: 457-481, 1958.

42. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39: 44-84, 2007.

43. Bjelland S and Seeberg E: Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. Mutat Res 531: 37-80, 2003.

44. Kryston TB, Georgiev AB, Pissis P and Georgakilas AG: Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 711: 193-201, 2011.

45. Schöttker B, Saum KU, Jansen EH, Boffetta P, Trichopoulou A, Holleczek B, Dieffenbach AK and Brenner H: Oxidative stress markers and all-cause mortality at older age: A population-based cohort study. J Gerontol A Biol Sci Med Sci 70: 518-524, 2015.

46. Schöttker B, Brenner H, Jansen EH, Gardiner J, Peasey A, Kubínová R, Pająk A, Topor-Madry R, Tamosiunas A, Saum KU, et al: Evidence for the free radical/oxidative stress theory of ageing from the CHANCES consortium: A meta-analysis of individual participant data. BMC Med 13: 300, 2015.

47. Lan Q, Zheng T, Shen M, Zhang Y, Wang SS, Zahm SH, Holford TR, Leaderer B, Boyle P and Chanock S: Genetic polymorphisms in the oxidative stress pathway and susceptibility to non-Hodgkin lymphoma. Hum Genet 121: 161-168, 2007.