Serum cell-free DNA concentration as a possible prognostic marker in newly diagnosed diffuse large B-cell lymphoma

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ABSTRACT
Cell-free DNA (cfDNA) is a fragment of DNA circulating in the blood, and its concentration is often elevated in cancer patients. To investigate the relationships between serum cfDNA concentration and clinical characteristics, including prognosis, we measured serum cfDNA concentration in 114 newly diagnosed lymphoma patients. The cfDNA concentrations in diffuse large B cell lymphoma (DLBCL) (62.5 ng/mL) and follicular lymphoma patients (51.6 ng/mL) were significantly elevated compared to healthy individuals (7.5 ng/mL, \( P < 0.001 \)). In DLBCL, patients with elevated serum cfDNA (> 38.9 ng/mL) at diagnosis had significantly shorter time-to-progression compared to those without (\( P = 0.033 \)). The addition of cfDNA concentration to the international prognostic index showed improved predictive power for time-to-progression. Moreover, cfDNA added significant prognostic value to other inflammatory markers such as B symptoms and sIL-2R. There was a trend towards shorter progression-free survival and overall survival in patients with elevated cfDNA. Furthermore, B symptoms (\( P = 0.038 \)), bulky masses (\( P = 0.031 \)), non-GCB subtype (\( P = 0.012 \)), and serum sIL-2R levels > 2,000 U/mL (\( P = 0.012 \)) were associated with higher cfDNA levels. Our study showed that serum cfDNA concentration at diagnosis was associated with certain clinicopathological characteristics, and may be predictive of survival outcomes in DLBCL patients.

INTRODUCTION
Cell-free DNA (cfDNA) is DNA fragments circulating in the blood, as first reported in 1948 (Mandel and Métais, 1948). It is released into the blood via apoptosis, necrosis, cell lysis, or extracellular vesicle secretion by tumor cells (Anker et al., 2001; Jahr et al., 2001; Stroun et al., 2001; Snyder et al., 2016). cfDNA can be found in serum or plasma of healthy individuals in small amounts, but its level is typically elevated in cancer patients compared to healthy individuals (Leon et al., 1977), including both lymphoid and myeloid haematological malignancies (Hohaus et al., 2009; Gao et al., 2010; Suzuki et al., 2016). Transient increase in cfDNA concentration after the initiation of chemotherapy in various types of malignancies has been reported (Tie et al., 2015; Kruger et al., 2018; Deshpande et al., 2021), while there are also some reports of an immediate decrease following treatment (Patsch et al., 2019; Moser et al., 2020). Associations between cfDNA level and prognosis have been studied in various types of solid tumors, including breast cancer (Gal et al., 2004), colorectal cancer (Lecomte et al., 2002; Hao et al., 2014; Siravegna and Bardelli,
2016), lung cancer (Sozzi et al., 2003; Ansari et al., 2016), pancreatic cancer (Chen et al., 2018) and prostate cancer (Bastian et al., 2007). Its usefulness as a diagnostic and prognostic biomarker has also been studied in haematological malignancy, including malignant lymphoma (Hohaus et al., 2009; Roschewski et al., 2015; Bohers et al., 2018). It has been suggested in several studies that cfDNA levels correlate with clinical characteristics of lymphoma patients, such as age, the international prognostic index (IPI), stage and lactate dehydrogenase (LDH) levels (Hohaus et al., 2009; Roschewski et al., 2015; Scherer et al., 2016; Bohers et al., 2018; Kurtz et al., 2018; Eskandari et al., 2019). Furthermore, cfDNA concentrations may be predictive of survival prognosis in patients with diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) (Delfau-Larue et al., 2018; Kurtz et al., 2018; Eskandari et al., 2019). Moreover, minimal residual disease assessment and disease monitoring using circulating tumor DNA assessment are being developed for FL (Galimberti et al., 2014; Nagy et al., 2020). Therefore, cfDNA has been studied as a non-invasive, more accessible tool to obtain clinical information in patients with cancer. Those studies mostly analyzed plasma to assess cfDNA concentration and clinical characteristics. However, obtaining and separating plasma requires an extra procedure. Serum is a more readily available form of sample, often routinely obtained in clinical practice. In this study, we used serum samples to analyze serum cfDNA concentration and its association with clinical characteristics in lymphoma patients, specifically DLBCL and FL.

MATERIALS AND METHODS

Patient population. Patients who were diagnosed with malignant lymphoma at our institution between March 2016 and March 2017 were enrolled in this study. The histological diagnosis was performed by haematopathologists at our institution, using biopsied specimens, in accordance with the revised fourth edition of the World Health Organization classification of Tumours of Haematopoietic and Lymphoid Tissues (Swerdlow et al., 2017). DLBCL was further divided into germinal center B-cell like (GCB) type and non-GCB type by Hans criteria (Hans et al., 2004). Peripheral blood samples from lymphoma patients were obtained at the time of initial diagnosis. Clinical data were obtained retrospectively.

Study design. Serum cfDNA concentration of lymphoma patients were compared to that of control (promeddx, MA, USA). Additionally, cfDNA concentrations in different lymphoma subtypes were each compared to that of control. Patients with mixed histology were excluded from this subtype analysis. We then specifically analyzed patients with DLBCL and FL for correlation between serum cfDNA concentration and clinical characteristics, time-to-progression (TTP), progression-free survival (PFS), and overall survival (OS). Response to treatment was evaluated using 18F-fluorodeoxyglucose positron emission tomography (PET), in accordance with the revised response criteria for malignant lymphoma (Cheson et al., 2014). TTP was defined as the time from initial diagnosis to disease progression or relapse. PFS was defined as the time from initial diagnosis to disease progression/relapse or death from any cause, and OS as the time from initial diagnosis to death from any cause. All participants provided written informed consent. This protocol was approved by the Institutional Review Board and the Genomic Review Board of the Japanese Foundation for Cancer Research.

Purification of cell-free DNA and sequences. Peripheral blood samples obtained at diagnosis were collected in a tube with a serum-separating agent (Thermo Fisher Scientific, Tokyo, Japan). After being centrifuged at 1200 rpm for 10 min, the serum was collected and stored at −80°C. The cfDNA was extracted from the serum using a Maxwell RSC cfDNA Plasma kit (Promega, Tokyo, Japan) according to the manufacturer’s protocol. The concentration of cfDNA was calculated by fluorometer. Serum samples of 10 healthy individuals (promeddx) were used as control.

Statistical analysis. The serum cfDNA level was compared between lymphoma patients and control using the Mann-Whitney U test. Further comparison for different lymphoma subtypes was adjusted for multiple comparisons using the Bonferroni method. TTP, PFS, and OS were estimated by the Kaplan-Meier method, and differences were compared using the log-rank test. Significance of cfDNA concentration analysis in TTP prediction was assessed by likelihood ratio test comparing a full model, including established risk factors and serum cfDNA concentration, to a reduced model not including serum cfDNA concentration. The relationships between cfDNA level and clinicopathological characteristics were assessed by the Mann-Whitney U test. All statistical analyses were performed using EZR (Kanda,
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2013), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). The level of significance was defined as \( P \leq 0.05 \).

RESULTS

cfDNA concentrations in different lymphoma subtypes

We identified and obtained serum samples from 114 patients with lymphoma. The median cfDNA concentration for patients with lymphoma was 56.6 ng/mL (range 6.2–1220.0 ng/mL), which was significantly higher than that for control (median 7.5 ng/mL, range 4.4–14.1 ng/mL; \( P < 0.001 \); Fig. 1). A breakdown of lymphoma subtypes included in the study is shown in Fig. 2A. Levels of cfDNA were significantly elevated in patients with DLBCL compared to control (\( n = 66 \), median 62.5 ng/mL, \( P < 0.001 \)), FL (\( n = 17 \), median 51.6 ng/mL, \( P < 0.001 \), and extranodal marginal zone B cell lymphoma (\( n = 8 \), median 37.4 ng/mL, \( P = 0.024 \)). Albeit not statistically significant due to small sample sizes, higher cfDNA levels were also observed in Burkitt lymphoma (\( n = 1 \), 136.0 ng/mL), Hodgkin's lymphoma (\( n = 4 \), median 116.0 ng/mL), mantle cell lymphoma (\( n = 3 \), median 25.3 ng/mL), and peripheral T cell lymphoma (\( n = 3 \), median 122.2 ng/mL, \( P = 0.15 \)) (Fig. 2B). Furthermore, no statistically significant differences in cfDNA concentration were detected among different lymphoma subtypes.

Association between serum cfDNA levels and patient characteristics in DLBCL and FL

Table 1 shows the correlation between serum cfDNA concentration at diagnosis, and clinicopathological characteristics of 63 patients with DLBCL. Among patients with DLBCL, B symptoms, bulky masses, non-GCB subtype, and serum sIL-2R levels higher than 2,000 U/mL were found to be associated with significantly higher cfDNA (\( P = 0.038, 0.031, 0.012, 0.012 \), respectively). From these data, we hypothesized that serum cfDNA may reflect aggressive tumor growth. We analyzed an association between

![Fig. 1](image1.png)

Comparison of cfDNA concentration between all lymphoma patients and healthy individuals. Patients with lymphoma had significantly higher cfDNA concentrations compared to control (Median concentration, 56.6 ng/mL in lymphoma patients vs. 7.5 ng/mL in control, \( P < 0.001 \)).

![Fig. 2](image2.png)

(A) Breakdown of histological subtypes, (B) cfDNA concentration of each lymphoma subtype compared to that of healthy control. Statistically significant elevation in cfDNA concentration was observed in DLBCL (\( n = 66 \), median 62.5 ng/mL, \( P < 0.001 \)), FL (\( n = 17 \), median 51.6 ng/mL, \( P < 0.001 \)), and MALT (\( n = 8 \), median 37.4 ng/mL, \( P = 0.024 \)), when compared with healthy individuals. No significant differences were observed between subtypes. * \( P = 0.024 \), ** \( P < 0.001 \).
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uximab, cyclophosphamide, doxorubicin, vincristine, and prednisone)-like regimens. FL patients were treated with either rituximab monotherapy or rituximab-containing immunochemotherapy. Using the ROC curve, a cfDNA concentration of 38.9 ng/mL was set as the cut-off value. With the median follow-up period of 40.8 months, we found that patients with elevated cfDNA (> 38.9 ng/mL) had significantly shorter TTP compared with patients whose cfDNA levels were not elevated, as shown in Fig. 3A (progression free rate at 3 years, 75.1% [95% confidence interval, 95% CI, 60.4–85.1%] vs. 100%, \( P = 0.033 \) by the log-rank test). Furthermore, we performed multivariable analysis to determine the prognostic value of serum cfDNA concentration on TTP. We found that cfDNA concentration showed significant incremental prognostic information when compared with the IPI (age > 60, elevated LDH, ECOG performance status > 1, stage III or IV, and extranodal sites > 1) in predicting TTP (likelihood ratio \( P = 0.044 \)). Moreover, cfDNA concentration

cfDNA, cell-free DNA; GCB, germinal center B-cell like; H, high; HI, high-intermediate; IPI, international prognostic index; IQR, interquartile range; L, low; LDH, lactate dehydrogenase; LI, low-intermediate; sIL-2R, soluble interleukin-2 receptor; UNL, upper normal limit.

### Table 1 Association between cfDNA levels and clinicopathological characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n = 63 (%)</th>
<th>Median cfDNA (IQR; ng/mL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age ≤ 60</td>
<td>18 (28.6)</td>
<td>51.7 (38.3 – 76.3)</td>
<td>0.398</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>45 (71.4)</td>
<td>63.3 (41.7 – 139.3)</td>
<td></td>
</tr>
<tr>
<td>bone marrow invasion +</td>
<td>8 (12.7)</td>
<td>63.1 (45.5 – 72.9)</td>
<td>0.757</td>
</tr>
<tr>
<td>−</td>
<td>55 (87.3)</td>
<td>61.7 (39.7 – 145.9)</td>
<td></td>
</tr>
<tr>
<td>B-symptoms +</td>
<td>10 (16.1)</td>
<td>100.3 (51.1 – 669.5)</td>
<td>0.042</td>
</tr>
<tr>
<td>−</td>
<td>52 (83.9)</td>
<td>56.64 (38.3 – 108.8)</td>
<td></td>
</tr>
<tr>
<td>bulky masses +</td>
<td>7 (11.1)</td>
<td>230.6 (147.2 – 370.5)</td>
<td>0.033</td>
</tr>
<tr>
<td>−</td>
<td>56 (88.9)</td>
<td>56.9 (39.7 – 107.9)</td>
<td></td>
</tr>
<tr>
<td>CD5 +</td>
<td>7 (11.1)</td>
<td>56.9 (44.9 – 92.9)</td>
<td>0.991</td>
</tr>
<tr>
<td>−</td>
<td>56 (88.9)</td>
<td>62.4 (40.3 – 140.4)</td>
<td></td>
</tr>
<tr>
<td>cell of origin</td>
<td>GCB</td>
<td>31 (49.2)</td>
<td>0.144</td>
</tr>
<tr>
<td>non-GCB</td>
<td>32 (50.8)</td>
<td>47.3 (34.6 – 81.4)</td>
<td></td>
</tr>
<tr>
<td>extranodal lesion ≤ 1</td>
<td>48 (76.2)</td>
<td>56.6 (38.3 – 113.7)</td>
<td>0.140</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>15 (23.8)</td>
<td>63.3 (49.2 – 201.5)</td>
<td></td>
</tr>
<tr>
<td>IPI H/HI</td>
<td>26 (41.3)</td>
<td>63.1 (45.6 – 151.0)</td>
<td>0.292</td>
</tr>
<tr>
<td>L/LI</td>
<td>37 (58.7)</td>
<td>56.3 (37.8 – 111.5)</td>
<td></td>
</tr>
<tr>
<td>Ki-67 &gt; 80%</td>
<td>6 (9.8)</td>
<td>141.5 (126.7 – 167.1)</td>
<td>0.083</td>
</tr>
<tr>
<td>≤ 80%</td>
<td>55 (90.1)</td>
<td>56.9 (40.1 – 101.3)</td>
<td></td>
</tr>
<tr>
<td>LDH &gt; UNL</td>
<td>≤ UNL</td>
<td>33 (52.4)</td>
<td>0.109</td>
</tr>
<tr>
<td>&gt; UNL</td>
<td>30 (47.6)</td>
<td>50.1 (36.0 – 111.5)</td>
<td></td>
</tr>
<tr>
<td>sex Female</td>
<td>36 (55.4)</td>
<td>61.7 (42.0 – 130.9)</td>
<td>0.972</td>
</tr>
<tr>
<td>Male</td>
<td>29 (44.6)</td>
<td>62.5 (39.3 – 126.1)</td>
<td></td>
</tr>
<tr>
<td>sIL-2R &lt; 2000 U/l</td>
<td>45 (71.4)</td>
<td>50.1 (37.8 – 83.1)</td>
<td>0.006</td>
</tr>
<tr>
<td>≥ 2000 U/l</td>
<td>18 (28.6)</td>
<td>114.1 (62.9 – 228.0)</td>
<td></td>
</tr>
<tr>
<td>stage I/II</td>
<td>38 (60.3)</td>
<td>54.9 (38.0 – 107.2)</td>
<td>0.182</td>
</tr>
<tr>
<td>III/IV</td>
<td>25 (39.7)</td>
<td>72.0 (45.2 – 175.0)</td>
<td></td>
</tr>
</tbody>
</table>

relationships between cfDNA concentrations and survival outcomes in patients with DLBCL and FL

We selected 63 patients with DLBCL and 17 patients with FL, whose clinical datasets were available, for the analysis of the relationship between serum cfDNA concentration and prognosis. All patients with DLBCL were treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone)-like regimens. FL patients were treated with either rituximab monotherapy or rituximab-containing immunochemotherapy. Using the ROC curve, a cfDNA concentration of 38.9 ng/mL was set as the cut-off value. With the median follow-up period of 40.8 months, we found that patients with elevated cfDNA (> 38.9 ng/mL) had significantly shorter TTP compared with patients whose cfDNA levels were not elevated, as shown in Fig. 3A (progression free rate at 3 years, 75.1% [95% confidence interval, 95% CI, 60.4–85.1%] vs. 100%, \( P = 0.033 \) by the log-rank test). Furthermore, we performed multivariable analysis to determine the prognostic value of serum cfDNA concentration on TTP. We found that cfDNA concentration showed significant incremental prognostic information when compared with the IPI (age > 60, elevated LDH, ECOG performance status > 1, stage III or IV, and extranodal sites > 1) in predicting TTP (likelihood ratio \( P = 0.044 \)). Moreover, cfDNA concentration

cfDNA, cell-free DNA; GCB, germinal center B-cell like; H, high; HI, high-intermediate; IPI, international prognostic index; IQR, interquartile range; L, low; LDH, lactate dehydrogenase; LI, low-intermediate; sIL-2R, soluble interleukin-2 receptor; UNL, upper normal limit.
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100% vs. 68.2% [95% CI, 47.7–82.0%], \( P = 0.26 \)) than those with GCB subtype (100% vs. 85.0% [95% CI, 60.4–94.9%], \( P = 0.187 \)) although the subgroup analyses were not powered to detect statistical significance. No correlation between cfDNA concentration and TTP was observed in patients with FL, with a median follow-up of 62.5 months (3-year progression free rate, 83.3% [95% CI, 27.3–97.5%] vs. 100%, \( P = 0.324 \)).

DISCUSSION

In this retrospective analysis, we showed that serum cfDNA concentration at diagnosis can be prognostic for time-to-progression (TTP) in patients with DLBCL, adding further prognostic value to other inflammatory markers (B symptoms and sIL-2R) as assessed by likelihood ratio test (\( P = 0.017 \)). Notably, even when all variables mentioned above were combined (the IPI, B-symptoms, and sIL-2R), addition of serum cfDNA concentration improved the prognostic power for TTP (likelihood ratio \( P = 0.047 \)). These results indicate that adding serum cfDNA to the established prognostic factors may allow a more robust prediction of TTP.

Patients with elevated serum cfDNA also trended towards a shorter PFS (3-year PFS, 71.1% [95% CI, 56.1–81.7%] vs. 92.9% [95% CI, 59.1–99.9%], \( P = 0.071 \), Fig. 3B) and OS (3-year OS, 83.1% [95% CI, 69.0–91.2%] vs. 92.9% [95% CI, 59.1–99.0%], \( P = 0.285 \), Fig. 3C), although the difference was not statistically significant. The difference in TTP was greater in patients with non-germinal center B-cell like (GCB) subtype (3-year progression free rate, 100% vs. 68.2% [95% CI, 47.7–82.0%], \( P = 0.26 \)) than those with GCB subtype (100% vs. 85.0% [95% CI, 60.4–94.9%], \( P = 0.187 \)) although the subgroup analyses were not powered to detect statistical significance. No correlation between cfDNA concentration and TTP was observed in patients with FL, with a median follow-up of 62.5 months (3-year progression free rate, 83.3% [95% CI, 27.3–97.5%] vs. 100%, \( P = 0.324 \)).

![Kaplan-Meier plots comparing time-to-progression (TTP; A), progression-free survival (PFS; B), and overall survival (OS; C) between patients with and without elevated cfDNA concentration (> 38.9 ng/mL). With a median follow-up period of 40.8 months, patients with elevated cfDNA (> 38.9 ng/mL) had a significantly shorter TTP (75.1% at 3 years) compared with those without elevated cfDNA levels (100% at 3 years, \( P = 0.033 \); A). There was a trend towards a shorter PFS (3-year PFS, 71.1% vs. 92.9%, \( P = 0.07 \); B) and OS (3-year OS, 89.7% vs. 100%, \( P = 0.285 \); C) in patients with elevated cfDNA, although the difference was not statistically significant.](image-url)
study is that serum samples may be used to predict survival as well as clinicopathological characteristics in this group of patients. Moreover, we revealed that certain characteristics such as B symptoms (including fever, weight loss, and night sweats), bulky masses, non-GCB subtype, and serum sIL-2R levels were associated with elevated serum cfDNA.

Increased concentration of cfDNA in lymphoma patients’ plasma has been reported in several studies (Hohaus et al., 2009; Roschewski et al., 2015; Bohers et al., 2018). Our results using serum samples were in line with those studies. In this study, we showed that serum cfDNA concentration also revealed tumor characteristics and predicted the prognosis of DLBCL patients. These findings have important clinical implications because serum samples are often obtained as part of routine blood tests, potentially eliminating the need for additional samples for cfDNA analysis.

Some clinicopathological characteristics we found to correlate with elevated serum cfDNA levels in DLBCL were in line with previous reports, such as bulky masses and B symptoms (Hohaus et al., 2009; Eskandari et al., 2019), while the others were newly reported in this study, such as sIL-2R levels (>2,000 U/ml) and non-GCB type. Serum sIL-2R level is reported to correlate with metabolic tumor volume in newly diagnosed DLBCL (Senjo et al., 2019). Combined with the fact that cfDNA levels were also correlated with the presence of bulky masses, this finding suggests that increased cfDNA is associated with high tumor burden in patients with newly diagnosed DLBCL. Additionally, we found a tendency for higher cfDNA concentration in patients with a high Ki-67 index (>80%), although no statistical difference was observed due to the small number of such patients. This suggests that elevated serum cfDNA concentration may reflect rapid tumor proliferation. Moreover, cfDNA is known to be elevated in autoimmune diseases (Tug et al., 2014; Rykova et al., 2017), and its levels have been reported to reflect disease activity (Abdelal et al., 2016). Combined with our findings that increased cfDNA was associated with B-symptoms and higher sIL-2R levels, it is possible that elevated serum cfDNA concentrations in those patients also reflects inflammatory reactions caused by rapid tumor growth. Of note, cfDNA concentration showed significant prognostic value when compared with other inflammatory markers such as B symptoms and sIL-2R.

In our analysis, elevated cfDNA at diagnosis was significantly associated with a shorter TTP in patients with DLBCL. Addition of serum cfDNA concentration to the established risk factors included in the IPI improved the prognostic power for TTP, suggesting substantial added value. The difference in TTP was greater in non-GCB subtype than GCB subtype. It is noteworthy that no TTP event occurred during the follow-up period in the group of patients whose serum cfDNA concentration at diagnosis was not elevated. Furthermore, association with progression-free survival (PFS) and overall survival (OS) was also suggested in DLBCL patients, but it was not statistically significant, possibly due to the small sample size. However, it was reported by a different group that elevated plasma cfDNA concentration at diagnosis was prognostic for OS in DLBCL (Eskandari et al., 2019). Hence, when making treatment decisions for newly diagnosed DLBCL patients, measuring cfDNA at diagnosis, in combination with other prognostic factors, can be beneficial in predicting survival outcomes. As a further possible treatment strategy, DLBCL patients with elevated cfDNA at diagnosis may require more intensive follow-up to detect early relapse.

No correlation between cfDNA levels and TTP was seen in FL patients. However, due to its indolent nature, further follow-up may be required to see more accurate results. Contrary to our results, a retrospective study that analyzed plasma cfDNA reported an adverse PFS in patients with elevated cfDNA at diagnosis (Delfau-Larue et al., 2018). Since data on the association between cfDNA and prognosis are limited, further study is required.

This study has several limitations. We used serum samples to perform cfDNA analysis, while previously published studies often used plasma samples. The lack of data on the association between serum and plasma cfDNA concentrations makes it difficult to compare the results of this study to the previously published data. Thus we used control serum samples to show that cfDNA levels are indeed elevated in lymphoma patients. Our results suggest that serum, which is more readily available than plasma, may be an appropriate alternative for cfDNA analysis. Moreover, the generalizability of this study is limited by the small sample size, and therefore, our findings need to be validated in a larger study with a longer follow-up period. Nevertheless, not only did we provide validation to published data that cfDNA concentration is elevated in lymphoma patients using serum samples, but we also established associations with certain clinicopathological characteristics in DLBCL. The next step is to investigate whether cfDNA can be used to monitor disease status after treatment in lymphoma patients.
In conclusion, the results of this study suggest that serum cfDNA concentration at diagnosis may be predictive of clinicopathological characteristics as well as survival outcomes in patients with DLBCL. This could help clinicians in treatment decision-making as well as survival predictions in this group of patients. Further research is warranted to better understand the nature of cfDNA in lymphoma, and to apply these data in clinical practice.

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CONFLICTS OF INTEREST

Yuko Shirouchi has received honoraria from Chugai Pharmaceutical Co. Ltd. Yuko Mishima has outsourced commissioned jobs from Chugai Pharmaceutical Co. Ltd., Roche Group, and received research funds from Eisai, Taiho, Kyowa-Kirin, Takeda pharmaceutical Inc, and Bristol Myers Squibb. Kengo Takeuchi has received grants from Fujibio and Daiichi Sankyo, royalties from Sysmex and Nichirei, consulting fees from Nichirei, Nippon Shinyaku, and Meiji, honoraria from Chugai, Kyowa Kirin, Takeda, Janssen, MDS, Eizai, Cellgene, Takult and Taiho. Dai Maruyama has received honoraria from Eisai, Kyowa Kirin, and Chugai Research, funding from Chugai, and scholarship donations from Eisai, Taiho, and Kyowa Kirin. The remaining authors declare no competing financial or other interests regarding this study.

REFERENCES


