# The derived neutrophil to lymphocyte ratio is an independent prognostic factor in patients with diffuse large B-cell lymphoma

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# BJC

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**Background:** With growing evidence on the role of inflammation in cancer biology, the systemic inflammatory response has been postulated as having prognostic significance in a wide range of different cancer types. Recently, the derived neutrophil to lymphocyte ratio (dNLR) has been proposed as an easily determinable prognostic factor in cancer patients. Nevertheless, its prognostic significance in diffuse large B-cell lymphoma (DLBCL) patients has never been explored.

**Methods:** Data from 290 consecutive DLBCL patients, diagnosed between 2004 and 2013 at a single Austrian centre, were evaluated retrospectively. The prognostic influence of the dNLR and other clinico-pathological factors including age, lactate dehydrogenase, cell of origin category and Ann Arbor stage on 5-year overall- (OS) and disease-free (DFS) survival was studied by Kaplan–Meier curves. To evaluate the independent prognostic relevance of dNLR, univariate and multivariate Cox regression models were applied.

**Results:** An independent significant association between high dNLR and poor clinical outcome in multivariate analysis for OS (HR = 2.02, confidence interval (CI) 95% = 1.17–3.50, $P = 0.011$), as well as DFS (HR = 2.15, CI 95% = 1.04–4.47, $P = 0.038$), was identified.

**Conclusion:** In the present study, we showed that a high dNLR at diagnosis of DLBCL represents an independent poor prognostic factor for clinical outcome. Our data encourage the further validation of this easily available parameter in prospective studies and as a potential stratification tool in clinical trials.

Diffuse large B-cell lymphoma (DLBCL) is the most commonly occurring form of lymphoma, accounting for 30–40% of newly diagnosed non-Hodgkin's lymphomas (NHL). With standard immunochemotherapy, DLBCL, even when in advanced stage, is considered a curable disease. Nevertheless, despite the improvements in therapy, approximately one-third of patients with advanced-stage DLBCL will still be refractory to therapy or will relapse (Friedberg, 2011).

Historically, clinicians and investigators have relied on prognostic schemes that imply clinical risk factors to predict the risk for disease progression, relapse and death of patients with aggressive NHL. One of the most commonly used schemes of rating, the International Prognostic Index (IPI) for lymphomas, developed in the 1990s, remains a robust clinical prognostic index for aggressive lymphomas. It involves five features: age, tumour stage, serum lactate dehydrogenase (LDH) concentration, performance status

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and number of extranodal disease sites. The IPI distinguishes four risk groups with different 5-year overall survival (OS), ranging from 26 to 73% (Shipp *et al*, 1993). In the era of Rituximab, a revised IPI (R-IPI) has been introduced, showing superior prediction in the outcome of DLBCL patients, treated with standard immunochemotherapy. The R-IPI identifies three distinct prognostic groups, with a very good (4-year OS 94%), good (OS 79%) and poor (OS 55%)) outcome, respectively (Sehn *et al*, 2007). Nevertheless, a large group of patients with distinct clinico-pathological profile and unfavourable outcome remains uncharacterized. Today, aside from clinical aspects, an additional distinction exists by the use of gene profiling, identifying two different subtypes, stratified by different survival times (Alizadeh *et al,* 2000). Genome-wide molecular profiling has revealed these subtypes of aggressive lymphoma, with tumour cells arising from different lymphoid maturation stages and usage of different oncogenic pathways. Patients with germinal center B-cell-like (GCB) DLBCL have a 5-year survival rate of 60%, as compared with a rate of less than 40% for patients with activated B-cell-like (ABC) DLBCL (Rosenwald *et al*, 2002). The crucial biological mechanisms that contribute to tumour development and further progression are not fully understood and more reliable and easy applicable prognostic factors, for individual risk assessment, have to be identified.

Inflammation has been identified to be a critical component of tumour progression, highlighting the role of the microenvironment, which is largely orchestrated by inflammatory cells as an indispensable participant in the neoplastic process, fostering proliferation, survival and migration (Coussens and Werb, 2002). For different solid tumours, as well as lymphomas, inflammation parameters, including leukocytes, neutrophils, lymphocytes and C-reactive protein, have been associated with higher mortality rates (Mohri *et al*, 2010; Cao *et al*, 2012).

In addition to absolute counts of inflammation parameters, also the neutrophil to lymphocyte ratio (NLR) has been identified as an independent prognostic factor for OS and progression free survival (PFS) in various types of cancer, including renal cell carcinoma, colorectal cancer, sarcoma and pancreatic cancer (Walsh *et al*, 2005; Zhang *et al*, 2012; Pichler *et al*, 2013a; Szkandera *et al*, 2013a). Recently, the NLR has been suggested to be a simple, inexpensive, standardized prognostic factor to assess clinical outcomes in DLBCL patients treated with R-CHOP (Porrata *et al,* 2010). Frequently, the absolute lymphocyte count is not routinely documented in clinical trials despite determining a differential white cell count. To solve this problem, the derived neutrophil to lymphocyte ratio (dNLR) was recently implemented, consisting of neutrophil count divided by (leukocyte count − neutrophil count) (Proctor *et al*, 2012). In this study by Proctor *et al* (2012), they proposed a similar prognostic value of the dNLR compared with the NLR in different solid cancer types but not explicitly for DLBCL patients. However, for hematological malignancies, the dNLR has not been validated yet. In the present study, therefore, we evaluated for the first time the prognostic significance of baseline dNLR in a large cohort of 290 patients, diagnosed with DLBCL.

## MATERIALS AND METHODS

This retrospective analysis included data from 290 consecutive patients who were diagnosed with DLBCL according to the 2008 World Health Organization (WHO) (Campo *et al*, 2011) criteria at the Division of Hematology at the Medical University of Graz between January 2004 and April 2013. All of the clinico-pathological data were retrieved from medical records from the Division of Hematology as well as from pathology reports from the

Institute of Pathology at the same institution. Clinico-pathological parameters included histologically confirmed DLBCL, gender, age, Ann Arbor stage and cell of origin categories (GCB and ABC subtype according to the Hans algorithm (Hans *et al*, 2004)). The laboratory data, including leukocyte, neutrophil and lymphocyte counts, were obtained by pre-diagnosis exploration 1–7 days before histologically proven diagnosis. Patients were treated by standard rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) regimen every 3 weeks for six to eight cycles. We do not routinely use combination radio-immunochemotherapy in early stage patients. According to the current ESMO guidelines, early stage lymphoma patients without bulky disease receive six cycles of R-CHOP every 3 weeks. Only patients with bulky disease are considered to treat with R-CHOP 21 × 6 with radiotherapy to the sites of previous bulky disease (Tilly *et al*, 2012). Post-treatment surveillance included routine clinical and laboratory examination. Regarding imaging methods, computer tomography was predominantly used. Follow-up evaluations were performed every 3 months during the first 5 years and annually thereafter. Patients were excluded in case of seropositivity of human immunodeficiency virus (HIV), missing laboratory parameters at diagnosis, lost to follow-up or central nervous system lymphoma. Dates of death were obtained from the central registry of the Austrian Bureau of Statistics or by telephone calls to their relatives. Overall survival was defined as the time (in months) from date of diagnosis until death due to any cause within the follow-up period. Disease-free survival was defined as the time (in months) from the date of the diagnosis to the date of demonstration of recurrent disease, confirmed radiologically or histologically. Disease-free survival was censored at the time of death or at the last follow up if the patients remained tumour free at that time. The study was approved by the local ethical committee of the Medical University of Graz.

**Statistical analyses.** The primary end point of the study was OS; the secondary end point was DFS. The optimal cutoff value for the dNLR was determined by applying receiver operating curve (ROC) analysis as previously described (Absenger *et al*, 2013). The cutoff value that discriminated best (in mean of sensitivity and specificity) between survival and death was used for OS. The cutoff value that discriminated best between disease-recurrence and no recurrence was used for DFS. The association between the dNLR with OS and DFS was analyzed using Kaplan–Meier curves and compared by the log-rank test. Backward stepwise multivariate Cox proportional analysis was performed to determine the influence of clinico-pathological variables, significantly associated with clinical outcome in univariate analysis of OS and DFS. Hazard ratios (HRs) and the corresponding 95% CIs were estimated from the Cox regression analysis. The assumption of proportional hazards was checked by LML-Plots and residual analysis using Schoenfeld plots. All statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA). A two-sided $P < 0.05$ was considered statistically significant.

## RESULTS

Overall, there were 137 (47.2%) male and 153 (52.8%) female patients diagnosed with DLBCL. The mean age at diagnosis was $65.5 \pm 15.5$ years. The Ann Arbor tumour stage was defined as stage I in 76 (26.2%) patients, stage II in 78 (26.9%) patients, stage III in 61 (21.0%) patients and stage IV in 75 (25.9%) patients. Extranodal disease was diagnosed in 128 (44.1%) patients. Eighty-nine patients featured histologically confirmed GCB subtype, 135 were identified as ABC subtype and 66 were unclassifiable/non-determined. Regarding the R-IPI, 21 (7.2%) had a very good R-IPI, 158 (54.5%) had a good R-IPI and 111 (38.3%) patients were
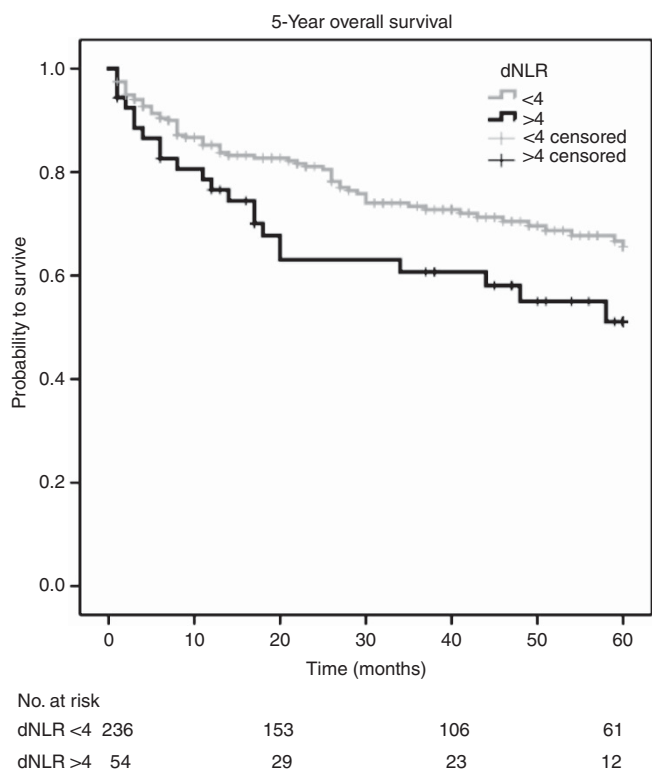
Figure 1. Kaplan–Meier curves for 5-year overall survival regarding high (⩾4) vs low (<4) dNLR ratio (P<0.043).

classified having a poor R-IPI. The median LDH was $255\,U1^{-1}$ (interquartile range: $191–412\,U1^{-1}$; upper limit of the normal range $200\,U1^{-1}$) and the mean dNLR was $2.94 \pm 1.95$ and the mean NLR was $5.52 \pm 4.78$. The Spearman rank correlation between the NLR and dNLR was 0.931 ($P<0.001$). Median follow-up was 33.5 months (interquartile range 10.7–60 months), 92 (31.7%) patients died and 69 (23.8%) had disease-recurrence by their most recent follow-up visit. Regarding the number of cycles of R-CHOP, we observed a significant lower number of cycles in early stage patients (5.1% of stage I patients received eight R-CHOP cycles vs 34% of stage II–IV patients, $P<0.001$) and in elderly patients (23.4% of patients <70 years received less than six cycles vs 45.7% of patients >70 years received less than six cycles, $P<0.001$).

First, we evaluated the previously published cutoff value (dNLR = 2) as the potentially optimal cutoff value for the continuous dNLR by the Kaplan–Meier curve analysis (Proctor et al, 2012). However, we could not find a survival difference between patients with low (<2) and high (⩾2) dNLR ($P=0.815$, data not shown). Therefore, applying the criteria mentioned above, we determined by ROC analysis a cutoff value of 4.0 for the dNLR to be best to discriminate between patients' survival and death in the whole cohort. This cutoff value prompted us to reevaluate the dNLR as a universally useful prognostic biomarker in our study cohort. Figure 1 shows the Kaplan–Meier curve for 5-year OS and reveals that a high dNLR (⩾4) is a consistent factor for poor prognosis in DLBCL patients ($P<0.047$, log-rank test). A high dNLR was not associated with gender, age, tumour stage, cell of origin or LDH levels ($P>0.05$, data not shown). Univariate Cox proportional analysis identified older age (<60 vs ⩾60, $P<0.001$),

**Table 1.** Univariate and multivariate Cox proportional of clinico-pathological parameters for the prediction of OS in patients with diffuse large B-cell lymphoma ($n=290$)

| Five-year OS | | | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|---|
| Parameter | No. at risk | No. events | HR (95% CI) | P-value | HR (95% CI) | P-value |
| **Age at diagnosis (years)** | | | | | | |
| <60 | 86 | 10 | 1 (Referent) | <0.001 | 1 (Referent) | 0.004 |
| ⩾60 | 204 | 75 | 3.82 (1.97–7.4) | | 3.52 (1.48–8.35) | |
| **Clinical stage (Ann Arbor)** | | | | | | |
| I and II | 154 | 31 | 1 (Referent) | <0.001 | 1 (Referent) | 0.012 |
| III and IV | 136 | 54 | 2.43 (1.56–3.78) | | 2.38 (1.21–4.68) | |
| **LDH** | | | | | | |
| Normal | 83 | 14 | 1 (Referent) | 0.003 | 1 (Referent) | 0.160 |
| $>200\,U1^{-1}$ | 204 | 71 | 2.42 (1.36–4.29) | | 1.62 (0.83–3.16) | |
| **Cell of origin** | | | | | | |
| GCB | 89 | 15 | 1 (Referent) | 0.002 | 1 (Referent) | 0.007 |
| non GCB | 135 | 50 | 2.49 (1.4–4.43) | | 2.25 (1.25–4.04) | |
| **dNLR** | | | | | | |
| <4 | 236 | 63 | 1 (Referent) | 0.047 | 1 (Referent) | 0.011 |
| ⩾4 | 54 | 22 | 1.64 (1.01–2.66) | | 2.03 (1.17–3.5) | |
| **Gender** | | | | | | |
| Female | 153 | 41 | 1 (Referent) | 0.544 | n.d. | n.d. |
| Male | 137 | 44 | 1.07 (0.86–1.32) | | | |

Abbreviations: CI = confidence interval; dNLR = derived neutrophil to lymphocyte ratio; GCB = germinal center B cell; HR = hazard ratio; n.d. = not done; No. at risk = number of individuals at risk; No. events = number of outcome events; OS = overall survival.
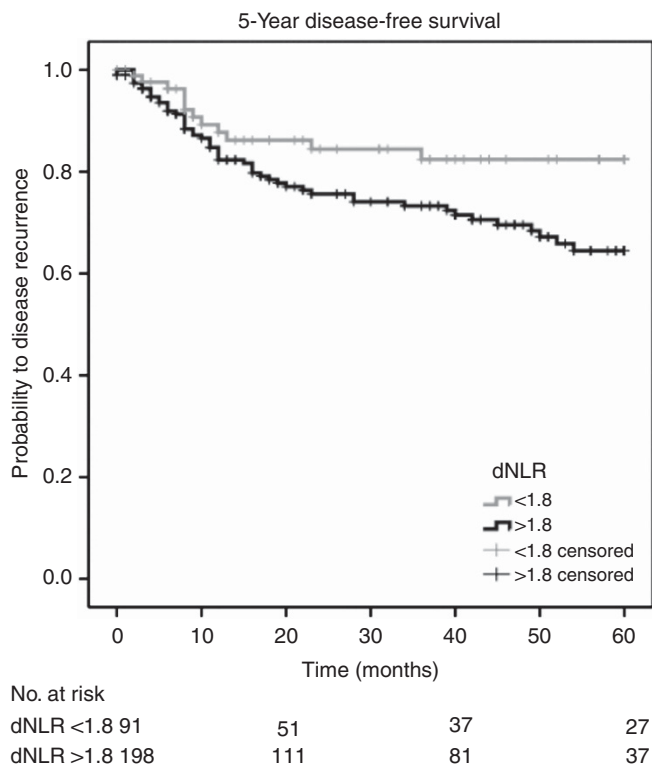
## 5-Year disease-free survival



| No. at risk | | | |
|---|---|---|---|
| dNLR <1.8 91 | 51 | 37 | 27 |
| dNLR >1.8 198 | 111 | 81 | 37 |

Figure 2. Kaplan–Meier curves for 5-year disease-free survival regarding high (⩾1.8) vs low (<1.8) dNLR ratio (P<0.032).

high tumour stage (Stage I + II vs stage III + IV, P<0.001), elevated LDH levels (normal vs >200 U l$^{-1}$, P=0.003), cell of origin (GCB vs ABC, P=0.002) and high dNLR (<4 vs ⩾4, P=0.047) as prognosticators of poor outcome for patients' OS, whereas gender and extranodal disease were not statistically significant associated with OS (Table 1).

To determine the independent prognostic value of the dNLR for OS, a multivariate analysis using a Cox proportional hazard model was performed. In the multivariate analysis, which included all independent parameters significantly associated with clinical outcome in univariate analysis (age, tumour stage, LDH, cell of origin and dNLR), we identified age (P=0.004), tumour stage (P=0.012), cell of origin (P=0.007) and high dNLR (P=0.011) as independent prognostic factors for OS, whereas elevated LDH was not significantly associated with OS (Table 1).

Regarding DFS, we calculated for the dNLR a cutoff value of 1.8 to be optimal to discriminate between DFS and recurrence state. Figure 2 shows the Kaplan–Meier curves for 5-year DFS and reveals that a dNLR ⩾1.8 is a significant factor for shorter 5-year DFS in DLBCL patients (P<0.032, log-rank test). To determine the independent prognostic significance of the new established cutoff value of dNLR for DFS, a multivariate Cox proportional hazard model including all parameters significantly associated with DFS in univariate analysis (see Table 2) was calculated. In the multivariate analysis, we identified age (P=0.028), tumour stage (P=0.013), cell of origin (P=0.008) and the dNLR (P=0.038) as independent prognostic factors for DFS (Table 2).

Finally, we found a weak but significantly negative correlation between dNLR and monocyte count (R= −0.136, P=0.021, Spearman correlation). We calculated for the monocyte count a

| Table 2. Univariate and multivariate analysis of clinico-pathological parameters for the prediction of DFS in patients with diffuse large B-cell lymphoma (n = 290) | | | | | | |
|---|---|---|---|---|---|---|
| **Five-year DFS** | | | **Univariate analysis** | | **Multivariate analysis** | |
| **Parameter** | **No. at risk** | **No. events** | **HR (95% CI)** | **P-value** | **HR (95% CI)** | **P-value** |
| **Age at diagnosis (years)** | | | | | | |
| <60 | 86 | 13 | 1 (Referent) | 0.02 | 1 (Referent) | 0.028 |
| ⩾60 | 204 | 51 | 2.06 (1.12–3.79) | | 2.48 (1.11–5.58) | |
| **Clinical stage (Ann Arbor)** | | | | | | |
| I and II | 154 | 24 | 1 (Referent) | 0.001 | 1 (Referent) | 0.013 |
| III and IV | 136 | 40 | 2.42 (1.46–4.02) | | 2.6 (1.22–5.54) | |
| **LDH** | | | | | | |
| Normal | 83 | 10 | 1 (Referent) | 0.004 | 1 (Referent) | 0.083 |
| >200 U l$^{-1}$ | 204 | 54 | 2.67 (1.36–5.25) | | 2.01 (0.91–4.43) | |
| **Cell of origin** | | | | | | |
| GCB | 89 | 12 | 1 (Referent) | 0.008 | 1 (Referent) | 0.008 |
| non GCB | 135 | 36 | 2.42 (1.26–4.66) | | 2.43 (1.26–4.67) | |
| **dNLR** | | | | | | |
| <1.8 | 91 | 12 | 1 (Referent) | 0.036 | 1 (Referent) | 0.038 |
| ⩾1.8 | 198 | 52 | 1.96 (1.04–3.67) | | 2.16 (1.04–4.47) | |
| **Gender** | | | | | | |
| Female | 153 | 28 | 1 (Referent) | 0.183 | n.d. | n.d. |
| Male | 137 | 36 | 1.18 (0.92–1.51) | | | |

Abbreviations: CI = confidence interval; DFS-disease-free survival; dNLR = derived neutrophil to lymphocyte ratio; GCB = germinal center B cell; HR = hazard ratio; n.d. = not done; No. at risk = number of individuals at risk; No. events = number of outcome events; OS = overall survival.

cutoff value of $700\,mm^{-3}$ as optimal for discrimination of OS. This cutoff value is very similar to a study by Tadmor et al (2013) ($630\,mm^{-3}$) who demonstrated a prognostic role for monocyte count in DLBCL patients. To test whether monocyte count is also an independent prognostic value in our cohort, we calculated a multivariate Cox model that also includes the monocyte count as a prognostic variable. Importantly, we found a statistically significant prognostic meaning for both, the dNLR ($P = 0.004$) and the monocyte count ($P = 0.038$, HR = 1.75 95% CI = 1.03–2.9), indicating that both parameters add independent prognostic information to well-established prognosticators.

## DISCUSSION

In this study, we validated for the first time the prognostic value of dNLR in a large cohort of DLBCL patients. Univariate analysis as well as multivariate analysis identified dNLR as a prognostic factor of 5-year OS and 5-year DFS.

In general, inflammatory processes have been identified as critical components of tumour progression (Coussens and Werb, 2002). Inflammatory cells can release growth and survival factors, promoting angiogenesis and lymphangiogenesis, stimulate DNA damage and promote tumour evasion of the host defense mechanisms (De Visser et al, 2006). Although the inflammatory response can be expected to have tumour suppressive actions, cancer patients often lack sufficient inflammatory response (Finn, 2012). In various types of cancers, for example, breast cancer, melanoma and lymphoma, innate immune cells like granulocytes, macrophages and mast cells correlate with increased angiogenesis and/or poor prognosis, which is in part explained by upregulation of cyclooxygenase-2 or suppression of anti-tumour adaptive immune response (Leek et al, 1996; Liu et al, 2001; Schoppmann et al, 2002; Dannenberg and Subbaramaiah, 2003; Ribatti et al, 2003). On the other hand, infiltrating lymphocytes are associated with favourable prognosis which was recently shown in non-small cell lung cancer and ovarian cancer (Sato et al, 2005; Horne et al, 2011). The adaptive immune cells such as B-lymphocytes, CD4 + helper T-lymphocytes and CD8 + cytotoxic T-lymphocytes modulate cancer development via cytokine-mediated lysis of tumour cells or establishing a pro-inflammatory state in the tumour micro-environment, revealing the paradoxical role of adaptive and innate leukocytes as crucial opposing regulators in cancer development (Ishigami et al, 2000; Zou, 2005).

The critical role of B-lymphocytes in initiating chronic inflammation during pre-malignancy has already been demonstrated by De Visser et al (2005). In a tumour-prone mouse model deficient in B and T cells, adoptive transfer of B-lymphocytes restores innate immune cell infiltration into pre-malignant tissue and reinstates necessary parameters for full malignancy. These findings support the hypothesis in which B-lymphocytes are required for establishing chronic inflammatory states that promote de novo carcinogenesis. Further, in a murine model, a subset of regulatory B-cells was recently found to inhibit anti-CD20 immunotherapy-mediated lymphoma depletion through the production of interleukin-10, a potent regulator of inflammation and autoimmunity. Even if present in small amount, they negatively influence effector functions of monocytes and in consequence, the lymphoma response to antibody-targeted therapy (Horikawa et al, 2011). However, despite the substantial progress and novel insights into lymphomagenesis during the past years, clinicians also require fast and easily measurable tools as indicators for patient survival. Within the last recent years, the systemic inflammatory response has been identified as an important driver of cancer progression in different types of cancer (Proctor et al, 2011a).

Different laboratory parameters including the modified Glasgow prognosis score (Proctor et al, 2011b), the neutrophil-lymphocyte ratio, the platelet-lymphocyte ratio, the C-reactive protein or fibrinogen levels (Pichler et al, 2013b) have been proposed as prognostic parameters that adequately reflect this systemic inflammatory response. However, there is plenty of clinical trial data, where only leukocyte and lymphocyte counts have been recorded. To overcome the lacking data, Proctor et al (2012) developed the dNLR and demonstrated the non-inferiority to the NLR in a large cohort of patients with different types of cancer. As the dNLR is mainly derived from the count of neutrophils and lymphocytes, our study also supports the potential of widespread use of this biomarker as a surrogate for inflammatory response.

Importantly, the variation over time and factors that might influence the dNLR have to be discussed. In general, the NLR (and also the dNLR) is supposed to reflect the systemic inflammatory response that accompanies chronic diseases, but might also be influenced by many different factors, including systemic infections, atherosclerosis, hypertension, chronic renal diseases and diabetes and can be even affected by atherosclerotic risk factors and drug treatment (Szkandera et al, 2013b). In our study, the previously published cutoff value of 2, as proposed in the study of Proctor et al (2012), showed no prognostic information in our cohort. The reasons for this discordance might be explained by the missing information in their study. In their original report about the dNLR, Proctor et al (2012) reported data from the Scottish cancer registry including hematological cancers. However, they did not separately analyze different hematological cancer entities. Moreover, they have no data for important prognostic variables like stage or others, which were included in the multivariate model in our study cohort. However, our study was not without limitations. Although we used a strategy to separately determine the optimal cutoff value for each end point as previously reported (Absenger et al, 2013), these cutoff values have to be externally validated in independent cohorts, most preferable in a prospective manner.

In conclusion, although it warrants further validation in independent prospective studies, the dNLR is an easily available and inexpensive marker in clinical studies and routine and has shown considerable potential as new prognostic marker for patients with DLBCL.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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