博士学位論文

ヘモグロビン値と血小板数に基づくびまん性大細胞型B細胞リンパ腫・非特定型の新たな予後予測指標の提唱
及び血球減少症とリンパ腫細胞におけるIL-6発現との関連性について

近畿大学大学院
医学研究科医学系専攻

谷口貴英

Doctoral Dissertation

Novel prognostic predictor of haemoglobin–platelet index in diffuse large B-cell lymphoma, not otherwise specified: Anaemia and thrombocytopenia are associated with IL-6 production in lymphoma cells

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Major in Medical Sciences
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Takahide Taniguchi

課博

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近畿大学大学院
医学研究科長　殿

共著者 [印]　　共著者 [印]

共著者 [印]　　共著者 [印]

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共著者 [印]　　共著者 [印]

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記

1．博士論文提出者氏名　　谷口　貴英

2．専攻分野　　医学系　　血液・免疫・腫瘍病／機能制御学

近畿大学大学院医学研究科

課博

同　意　書

令和4　　年　　5 月　　13 日

近畿大学大学院
医学研究科長　殿

共著者　賴晋也　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

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課博

同　意　書

令和4　　年　5 月　13 日

近畿大学大学院
医学研究科長　殿

共著者　橋本重夫　㊞　　共著者　松田光弘　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

共著者　＿＿＿＿＿＿＿＿　㊞　　共著者　＿＿＿＿＿＿＿＿　㊞

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2．専攻分野　　医学系　　血液・免疫・膠原病／機能制御学

Novel prognostic predictor of haemoglobin -platelet index in diffuse large B-cell lymphoma, not otherwise specified: anaemia and thrombocytopenia are associated with IL-6 production in lymphoma cells

Takahide Taniguchi M.D.[1], Shoko Nakayama M.D., Ph.D.[1], Hirokazu Tanaka M.D., Ph.D.[1], Shinya Rai M.D., Ph.D.[1], Chikara Hirase M.D., Ph.D.[1], Yasuyoshi Morita M.D., Ph.D.[1], Yoichi Tatsumi M.D., Ph.D.[1], Takashi Ashida M.D., Ph.D.[1], Mitsuhiro Matsuda M.D., Ph.D.[2], Shigeo Hashimoto M.D., Ph.D.[3], and Itaru Matsumura M.D., Ph.D.[1]

[1]Division of Hematology and Rheumatology, Faculty of Medicine, Kindai University Hospital, 377-2, Onohigashi, Osakasayama-shi, Osaka 589-8511, Japan
[2]Department of Hematology, Perfect Liberty General Hospital, 2204, Shindo, Tondabayashi-shi, Osaka 584-8585, Japan
[3]Department of Pathology, Perfect Liberty General Hospital, 2204, Shindo, Tondabayashi-shi, Osaka 584-8585, Japan

Running Title: HAEMOGLOBIN-PLATELET INDEX TO PREDICT PROGNOSIS OF DLBCL, NOS

Correspondence to:
Shoko Nakayama, M.D., PhD.
Division of Hematology and Rheumatology
Faculty of Medicine, Kindai University Hospital,
377-2, Onohigashi, Osakasayama-shi, Osaka 589-8511, Japan
Phone: +81-72-366-0221; Fax: +81-72-368-3732;
E-mail: s.nakayama@med.kindai.ac.jp

Abstract

We previously reported a novel haemoglobin-platelet index (HPI) based on anaemia and thrombocytopenia was useful to predict the prognosis of the patients with diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS). Here, we analysed the utility of HPI in a new validation cohort with DLBCL, NOS (n=94). As a result, we confirmed that HPI was effective to differentiate the progression-free survival (PFS) and overall survival in this validation cohort. So, we further compare the utility of HPI with previously reported prognostic markers such as NCCN-IPI, Glasgow prognostic score (GPS), and platelet-albumin (PA) score, using a larger number of 160 patients consisting of the derivation cohort (n=66) and validation cohort (n=94). As a result, the patients with a higher HPI score had significantly worse outcomes, and HPI predicted the prognosis of DLBCL, NOS independently of NCCN-IPI. HPI was more senisitive than GPS and almost the same as PA score to predict PFS. Moreover, the patients whose lymphoma cells were positive for interleukin-6 (IL-6) (75/111 cases) judged by the immunohistochemical staining had significantly lower haemoglobin levels and platelet counts than IL-6-negative cases (36/111 cases), suggesting the involvement of IL-6 produced by lymphoma cells in anaemia and thrombocytopenia in DLBCL, NOS patients.

Introduction

Diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) is the most common type of non-Hodgkin lymphoma, including heterogeneous subgroups in terms of clinical features, histologies, and molecular abnormalities (1). The International Prognostic Index (IPI) has been the most broadly used prognostic marker, which was based on the clinical outcomes of DLBCL patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone). However, rituximab has greatly improved the clinical outcomes of DLBCL patients (2). In 2004, the National Comprehensive Center Network (NCCN) published a modified NCCN-IPI (3), which discriminates low- and high-risk DLBCL patients better than IPI in the rituximab era. However, NCCN-IPI is not still enough to accurately identify patients who will not be benefitted from R-CHOP therapy (3). In addition, several molecular prognostic markers based on mutational and/or gene expression profiles have been developed (4, 5). However, it is difficult to apply these molecular markers in daily practice. Also, although the utility of immunohistochemical analysis (6-9) was demonstrated, it is time-consuming and requires laboratory tasks with high technical skills.

Thus, a more convenient prognostic marker had been hoped. To address this issue, several clinical markers using haematologic parameters such as lymphocyte/monocyte ratio (LMR) (10), neutrophil/lymphocyte ratio (NLR) (11), and platelet/lymphocyte ratio (PLR) (12) had been proposed. In addition, inflammatory and nutritional parameters had been utilized in combination with haematologic parameters as follows: platelet-albumin (PA) score (13), prognostic index (PI) (using C-reactive protein [CRP] levels and white blood cell [WBC] counts) (14), prognostic nutritional index (PNI) (calculated from serum albumin and total lymphocyte count) (15). Most of these markers were originally established to predict the prognosis of the patients with solid cancers. However, their usefulness was subsequently confirmed in DLBCL, NOS (16). Furthermore, Glasgow prognostic score (GPS) combining

CRP and albumin levels was shown to be superior to these inflammation-based scores (PI, NLR, PNI and PLR) to predict survival of DLBCL, NOS patients (16, 17).

We previously showed anaemia and thrombocytopenia were both poor prognostic markers of overall survival (OS) in 89 DLBCL, NOS patients treated with R-CHOP. Based on this result, we proposed a novel prognostic parameter, haemoglobin-platelet index (HPI) for DLBCL, NOS patients (18).

Here, we evaluated its utility in a new 94 DLBCL, NOS patients as a validation cohort. In addition, we compared it with previously reported prognostic markers, using a larger number of 160 patients including both previous and validation cohorts. In addition, we examined the association between anaemia or thrombocytopenia and the production of interleukin-6 (IL-6) by lymphoma cells themselves, because it was reported to be associated with anaemia in DLBCL (19).

Materials and Methods

Patients and treatment

We retrospectively analysed 160 patients with nodal DLBCL, NOS treated with R-CHOP (2) as the 1st-line therapy at Kindai University Hospital (Osakasayama-shi, Osaka, Japan) or Perfect Liberty General Hospital (Tondabayashi-shi, Osaka) between 2012 and 2020. Among 160 patients, 66 patients were analysed in our previous study (18). So, the remaining 94 patients were newly analysed as a validation cohort. Then, we conducted further analyses using a total 160 patients consisting of both cohorts. After R-CHOP therapy, 45 patients received salvage therapies including R-MECP (20), R-ESHAP (21), R-GDP (22), R-GCD (23), R-Hyper-CVAD/MA (24), R-EPOCH (25), R-HD-MTX (26), B-R (27), R-VNCOP-B (28), R-FD (29), and radiation.

Staging was performed by fluorodeoxyglucose positron emission tomography/computed

tomography and bone marrow (BM) biopsy. None of the patients had detectable inflammatory disease or a bleeding disorder. The following clinical data at diagnosis were retrieved from the medical records within a week around the BM biopsy: age, sex, Ann Arbor clinical stage, the number of extranodal lesions, BM involvement, serum lactate dehydrogenase (LDH) concentration, WBC count, haemoglobin (Hb) level, platelet count, albumin level, and CRP level. NCCN-IPI, GPS, and PA score were calculated as reported previously (13, 17, 30). The criteria for anaemia and thrombocytopenia utilized in HPI were as follows: Hb < 13 g/dL for male and Hb < 12 g/dL for female (31) and platelet count < 100×10^9/L (13). HPI was calculated by assigning 1 point for anaemia and thrombocytopenia, respectively. Patients were divided into three groups: a high-risk group with score 2, an intermediate-risk group with score 1 and a low-risk group with score 0. Also, leukocytopenia was defined as WBC count < 4.0×10^9/L in this study.

This study was approved by the institutional review boards of the ethics committee at Kindai University Hospital (No. R02-064) and Perfect Liberty General Hospital (No. 8-20-208) and conducted according to the Declaration of Helsinki.

Histopathology and immunostaining

Lymph node biopsy samples were fixed in 10% formalin and embedded in paraffin. Sliced sections were subjected to haematoxylin and eosin staining and immunohistochemical staining with an EnVision system kit (Dako, Glostrup, Denmark), using the following primary antibodies (Abs): anti-CD3 (PS1; Leica Biosystems, Newcastle upon Tyne, UK), anti-CD10 (56C6; Nichirei, Tokyo, Japan), anti-CD20 (L26; Roche Diagnostics, Mannheim, Germany), anti-B-cell lymphoma 6 (BCL6) (PG-B6p; Dako), B-cell lymphoma 2 (BCL2) (124; Dako), anti-mutated myeloma-associated antigen 1 (MUM1) (MUM1p; Dako), anti-Ki-67 (MIB-1; Dako), and anti-MYC (Y69; Abcam, Cambridge, UK) Abs. Cases with ≥ 30% positive cells in

lymphoma cells were judged as positive for CD10, BCL6, and MUM1, ≥ 50% for BCL2, and ≥ 40% for MYC, respectively (8). Co-expression of MYC and BCL2 was considered as double-expressor lymphoma (1). According to the report by Hans CP *et al*. (8), lymphoma samples positive for CD10 or BCL6 and negative for MUM1 were judged as a germinal center B-cell (GCB) type and any other staining patterns were judged as a non-GCB type. The proportion of Ki-67-positive cells in total lymphoma cells was calculated as a Ki-67 index. Cytoplasmic reactivities ≥ 20% of the lymphoma cells were judged as positive for IL-6 expression (18) using the anti-IL-6 Ab (10C12; Leica Biosystems). Histologic sections were independently examined by two pathologists.

Statistical analyses

OS was defined as the interval from the start of treatment to the date of death from any causes. Subjects lost to follow-up were treated as censored at the last known date alive. Progression-free survival (PFS) was calculated from the beginning of the treatment until relapse, disease progression, or death from any causes whichever comes earlier. Subjects lost to follow-up were censored at the last known date alive without relapse. OS and PFS were estimated by the Kaplan-Meier method, and statistical comparisons were made with the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards regression model and the Wald test. A Pearson's chi-square test and the *t*-test were used to assess differences among groups. A correlation coefficient was calculated to evaluate associations between two variables and p values < 5% were considered statistically significant. Statistical analyses were performed using JMP version14 software (SAS Institute, Inc., Cary, NC, USA).

Results

Revaluation of HPI in a new validation cohort

At first, we evaluated whether HPI was useful to predict the prognosis of DLBCL, NOS, using new 94 patients as a validation cohort. The characteristics of these 94 patients at diagnosis are summarised in Table 1. In a new validation cohort, HPI effectively discriminate PFS and OS of low-, intermediate-, and high-risk groups (both $p < 0.0001$) (Fig. 1A and 1B).

Clinical characteristics of the total cohort

Because HPI was confirmed to be useful to predict prognosis of DLBCL, NOS patients, we compared it with previously reported prognostic markers, using the total cohort consisting of 66 patients in the previous derivation cohort and 94 patients in the validation cohort.

Table 1 summarises the characteristics of 160 patients at diagnosis. Mean age was 70.1 (range: 28 to 98) years old. Ninety-six patients (60%) were male and 64 patients (40%) were female. Twenty patients (13%) were with Ann Arbor stage I, 34 patients (21%) with stage II, 34 patients (21%) with stage III, and 72 patients (45%) with stage IV. According to the NCCN-IPI categorization, 9 patients (5%) were classified into a low-risk group, 43 patients (27%) into a low-intermediate-risk (LI) group, 67 patients (42%) into a high-intermediate-risk (HI) group, and 41 patients (26%) into a high-risk group, respectively. As for the cell origin, 60 patients (38%) were classified as a GCB type, 77 patients (48%) as a non-GCB type and the remaining 23 patients (14%) as unknown. Ninety-nine patients (65 male and 34 female) (62%) were judged as having anaemia and 13 patients (8%) as thrombocytopenia according to HPI criteria.

Relationship between haematologic parameters/HPI and other variables at baseline

At first, we examined the relationship between haematologic parameters and other clinical

variables such as sex, age, clinical stages, NCCN-IPI, and BM involvement at baseline. As shown in Table 2, there was no significant difference in these variables between the patients with and without leukocytopenia. Meanwhile, age of patients at diagnosis and clinical stages were significantly higher in patients with anaemia than those without anaemia. Also, patients with anaemia were more frequently grouped into NCCN-IPI high-risk group than those without anaemia. Similarly, there was a significant difference in the frequency of NCCN-IPI high-risk group between the patients with and without thrombocytopenia. In addition, BM involvement was more often complicated with thrombocytopenia than those without BM involvement, while it didn't influence anaemia or leukocytopenia. We also analysed the relationship between HPI and other variables. According to HPI, 60 patients (38%) were classified as a low-risk group; 88 patients (55%) as an intermediate-risk group; 12 patients (7%) as a high-risk group. Age, clinical stage, and NCCN-IPI were significantly higher in an HPI high-risk group than in HPI low- or intermediate-risk group. There was no significant difference in gender and regimen of the salvage therapy for relapsed/resistant lymphoma among three HPI risk groups (Table 2).

Clinical outcomes by haematologic parameters and HPI groups

All these 160 patients were treated with R-CHOP. PFS rates at 1, 3, and 5 years were 69.8%, 58.4%, and 51.9%, respectively (Fig. 2A). Also, OS rates at 1, 3, and 5 years were 80.6%, 75.0%, and 66.7%, respectively (Fig. 2B). These rates were consistent with the previous reports (32, 33).

We next analysed PFS and OS by HPI groups in a total cohort. Largely in agreement with the results of the validation cohort (Fig. 1A and 1B), HPI could effectively discriminate PFS and OS in accord with the classification of the risk groups (both $p < 0.0001$) (Fig. 2C and 2D). We analysed the influence of haematologic parameters at baseline on clinical outcomes. There

was no survival difference between the patients with and without leukocytopenia (data not shown). On the other hand, patients with anaemia showed significantly worse PFS and OS than those without anaemia ($p < 0.0001$ for PFS; $p = 0.0007$ for OS) (Fig. 2E and 2F). Also, Patients with thrombocytopenia showed a significantly worse PFS and OS compared with those without thrombocytopenia ($p < 0.0001$ for both PFS and OS) (Fig. 2G and 2H).

Clinical outcomes by NCCN-IPI risk groups

We also analysed the utility of NCCN-IPI in the total cohort. As shown in Fig 3A and 3B, NCCN-IPI effectively discriminated PFS and OS of each risk group (both $p < 0.0001$), indicating that NCCN-IPI is applicable to our cohort.

Significance of HPI and NCCN-IPI as prognostic factors

When HPI was compared with NCCN-IPI, high-risk patients in HPI experienced earlier disease progression and/or death than those in NCCN IPI (PFS at 1 year: HPI 31.2% *vs*. NCCN-IPI 39.2%; OS at 1 year: HPI 31.2% *vs*. NCCN-IPI 42.5%). In addition, HPI high-risk patients revealed poorer prognosis than that in the NCCN-IPI high-risk patients (PFS and OS at 5 years: HPI 0% and 0% *vs*. NCCN-IPI 31.3% and 38.7%). The univariate Cox regression analysis indicated that both HPI and NCCN-IPI were significant predictors of PFS and OS (Table 3). So, we applied a bivariate Cox regression analysis to test whether HPI was a prognostic factor independently of NCCN-IPI. As a result, we found that HPI could predict both PFS and OS independently of NCCN-IPI (Table 3).

Comparison of HPI with previously reported other prognostic scores (GPS and PA score)

We also compared HPI with previously reported prognostic scores using an univariate Cox regression analysis. Accordingly, the *p* value of HPI was smaller than that of GPS, and almost

the same as PA score to predict PFS (HP index $p < 0.0001$, GPS $p = 0.0137$, and PA score $p < 0.0001$) and OS (HPI $p < 0.0001$, GPS $p = 0.0311$, and PA score $p < 0.0001$), indicating that, at least in our cohort, HPI was an efficient and simpler predictor of both PFS and OS.

Significance of haematopoietic parameters as prognostic factors

To analyse the significance of the haematopoietic parameters as prognostic factors, we performed a univariate Cox proportional hazards regression analysis. As shown in Table 4, anaemia and thrombocytopenia were individually significant predictors of poor PFS (anaemia: relative risk [RR], 3.37; 95% confidence interval [CI], 1.79 to 6.35; $p = 0.0002$, and thrombocytopenia: RR, 3.94; 95% CI, 1.99 to 7.80; $p < 0.0001$) and OS (anaemia: RR, 4.03; 95% CI, 1.68 to 9.66; $p = 0.0018$, and thrombocytopenia: RR, 7.56; 95% CI, 3.63 to 15.75; $p < 0.0001$), whereas leukocytopenia was not a prognostic factor for PFS (RR, 1.11; 95% CI, 0.45 to 2.79; $p = 0.817$) or OS (RR, 1.19; 95% CI, 0.42 to 3.41; $p = 0.743$). Also, a multivariate Cox regression analysis showed that anaemia and thrombocytopenia were independent predictors of poor PFS and OS.

Relationship Hb levels and platelet counts correlated inversely with CRP levels and directly with albumin levels.

To further delineate clinical characteristics of the patients with anaemia and/or thrombocytopenia, we analysed their relationships with the levels of CRP and albumin. As a result, Hb levels showed a significant positive correlation with albumin levels (correlation coefficient, 0.5709; 95%CI, 0.4562 to 0.6670; $p < 0.0001$) and a negative correlation with CRP levels (correlation coefficient, −0.4416; 95%CI, −0.5585 to −0.3075; $p < 0.0001$). Platelet counts also showed a significant positive correlation with albumin levels (correlation coefficient, 0.1632; 95%CI, 0.0083 to 0.3105; $p = 0.0392$) and tendency to be a negative

correlation with CRP levels (correlation coefficient, −0.1071; 95%CI, −0.2580 to 0.0489; p = 0.1777).

Expression of IL-6 by lymphoma cells in DLBCL, NOS

IL-6 was reported to regulate CRP positively and albumin levels negatively (34) and to be involved in anaemia in various types of malignancies. Thus, we speculated that IL-6 produced by lymphoma cells might be involved in anaemia and/or thrombocytopenia in DLBCL, NOS patients. We examined the expression of IL-6 by lymphoma cells with immunohistochemical staining using available samples from 111 cases. Representative positive and negative photomicrographs of the staining patterns are shown in Fig. 4A and 4B, respectively. With cytoplasmic reactivities 20% as a positive cut-off value (18), lymphoma cells were positive for IL-6 (IL-6-positive cases) in 75 cases (68%) and negative (IL-6-negative cases) in 36 cases (32%).

Characterisation of IL-6-positive DLBCL

Next, we tried to characterise IL-6-posisitve cases. As summarised in Table 5, IL-6-positive cases were more frequently classified as non-GCB type compared with IL-6-negative cases with a significant difference (69% *vs*. 35%, p = 0.001). Meanwhile, IL-6 expression by lymphoma cells didn't show significant relationship with Ki-67 index, MYC expression, or biologic phenotype.

In addition, we analysed the relationship between IL-6 production by lymphoma cells and HPI risk groups. As a result, IL-6-positive cases were 22/75 (29%), 45/75 (60%), and 8/75 (11%) in HPI low-, intermediate-, and high-risk group, respectively, all of which were without a significant difference (Table 5).

Anaemia and thrombocytopenia associated with IL-6 positivity in lymphoma cells

Finally, we compared haematologic parameters between IL-6-positive and -negative cases. As shown in Fig. 4C, Hb levels were significantly lower in IL-6-positive cases (mean ± standard error [SE]: 113.54 ± 2.23 g/L) than those in IL-6 -negative cases (mean ± SE: 128.52 ± 3.22 g/L) (p = 0.0002, t-test). Similarly, platelet counts were significantly lower in IL-6-positive cases (mean ± SE: 219.21 ± 12.48 /L) than those in IL-6–negative cases (mean ± SE: 278.80 ± 18.02 /L) (p = 0.0076, t-test) (Fig. 4D). Because thrombocytopenia was accompanied by BM involvement (Table 2), we examined the platelet counts by the patients with or without BM involvement; in the patients without BM involvement, platelet counts were significantly lower in IL-6-positive cases than in IL-6-negative cases (Fig. 4E). Also, in the cases with BM involvement, platelet counts showed tendency to be lower in IL-6-positive cases than IL-6-negative cases, but was not significant (Fig. 4F). Meanwhile, there was no association between the WBC counts and the IL-6 expression by lymphoma cells (p = 0.8195, t-test).

Discussion

In accord with our result, some previous studies demonstrated anaemia was associated with poor clinical outcomes in DLBCL patients (35, 36), which was denied in another study (37). As for this reason, these studies ignored the gender difference (31) and set the same Hb cutoff value to define anaemia regardless of the sex (35-37). So, in this study, we utilized the cutoff value of anaemia according to the WHO criteria (31). Meanwhile, thrombocytopenia was reported to be a worse prognostic factor of PFS and OS in patients with DLBCL, NOS (13, 38), which was confirmed in our study. BM infiltration of lymphoma cells can be the reason for anaemia and/or thrombocytopenia in DLBCL, NOS (35, 37). However, these findings were denied in other studies (19, 36, 38, 39). In our analysis, we found that thrombocytopenia but not anaemia was associated by BM involvement. These inconsistent findings might result

from the different cutoff values of BM involvement of lymphoma cells.

We here found that Hb levels were positively correlated with albumin levels but inversely correlated with CRP levels; platelet counts also showed a positive correlation with albumin levels and tendency to be a negative correlation with CRP levels, suggesting that some systemic factor might be involved in anaemia and thrombocytopenia in DLBCL, NOS. Because IL-6 elevates CRP levels and reduce albumin level through reciprocal regulation of NF-IL-6 and C/EBP in liver cells, we speculated that IL-6 might be a causative molecule of anaemia and thrombocytopenia in DLBCL, NOS. IL-6 induces hepcidin in liver cells, which blocks the iron release from macrophages and enterocytes, thereby interfering with the supply of iron for erythropoiesis (40, 41). In fact, a previous study demonstrated that IL-6 levels were corelated with hepcidin levels and inversely correlated with Hb levels in DLBCL patients (19). Furthermore, we here showed that Hb levels were significantly lower in IL-6-positive cases than in IL-6-negative cases, suggesting that anaemia may be caused by IL-6 produced by the lymphoma cells themselves.

Although IL-6 is known to stimulate platelet production (42), platelet counts were significantly lower in IL-6-positive cases than in IL-6-negative cases in the patients without BM involvement. Meanwhile, in the patients with BM involvement, platelet counts showed tendency to be lower in IL-6 positive cases than in IL-6 negative cases, but was not significant. These results suggested that IL-6 produced by lymphoma cells might, at least in part, contribute to thrombocytopenia, which may be enhanced by BM involvement of lymphoma cells.

Moreover, there was no significance between HPI risk groups and IL-6 positive cases, which might due to the small numbers of cases categorised into each HPI risk group. Alternatively, this inconsistence might be caused by lack of measuring the amount of IL-6 production from whole lymphoma legions. Unfortunately, we couldn’t measure serum IL-6

levels due to the lack of serum samples in this study. However, high serum levels of IL-6 were reported to predict poor outcome in diffuse large B-cell lymphoma (43, 44). So, we need to examine the prognostic relevance of IL-6 production by lymphoma cells considering the amount of IL-6 production from all lymphoma legions, including serum IL-6 levels in the next study. In addition, IL-6 is mainly produced by macrophages rather than lymphocytes. However, it was reported that ABC type DLBCL cells produces IL-6 through high NF-κB activity, which is caused by genetic alterations of MYD88 involved in the Toll-like receptor and B cell receptor signalling. In this type of DLBCL, IL-6 was shown to act as an autocrine growth factor, leading to constitutively activation of JAK1 and STAT3 to promote cell survival (45). So, further studies based on the genetic mutation profiles would clarify the mechanism of IL-6 production by lymphoma cells and its role in the pathogenic features of DLBCL, NOS.

We previously proposed the utility of HPI as a prognostic index in DLBCL, NOS and its usefulness was confirmed in both a validation cohort with 94 patients and a total cohort with 160 patients in this study. The patients with a higher HPI score had significantly worse outcomes in both PFS and OS, suggesting a necessity of new treatment besides R-CHOP therapy. Because HPI and NCCN-IPI are independent prognostic factors, combining HPI with NCCN-IPI might be useful to predict the prognosis of the patients with DLBCL, NOS more accurately.

In conclusion, we here confirmed that HPI was a useful marker to predict the prognosis of the patients with DLBCL, NOS in the validation cohort and total cohort. In addition, we found that anaemia and thrombocytopenia complicated in DLBCL, NOS patients, might be caused by IL-6 production by lymphoma cells themselves.

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Authors' contributions

S.N. and I.M. designed and supervised the research. T.T., S.N., H.T., S.R., C.H., Y.M., Y.T., T.A., M.M., and I.M. collected clinical data. S.N. and T.T. performed statistical analysis. T.T., S.N., and I.M. performed the research and prepared figures and tables. T.T., S.N., S.H., performed immunostaining. S.N., and I.M. wrote the manuscript. All authors read and approved the manuscript.

Competing interests

Statement of conflict of interest

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Other authors declare that they have no competing interests.

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Figure Legends

Figure 1

Progression-free survival (PFS) (A) and overall survival (OS) (B) curves by haemoglobin-platelet index (HPI) risk groups in 94 patients with diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) as a validation cohort. Patients with a higher HPI score significantly had both worse outcomes.

Figure 2

PFS (A) and OS (B) curves patients with DLBCL, NOS in all 160 patients as a total cohort. PFS and OS curves by HPI, haemoglobin (Hb) levels, and platelet counts in patients with DLBCL, NOS. Patients with a higher HPI score significantly had both worse PFS (C) and OS (D). Patients with anaemia had significantly worse PFS (E) and OS (F) than those without anaemia. Those with thrombocytopenia had a significantly worse PFS (G) and OS (H) than those without thrombocytopenia.

Figure 3

PFS (A) and OS (B) by the NCCN-IPI category in patients. A higher NCCN-IPI score was associated with worse outcomes.

Figure 4

Representative results of the immunohistochemical staining of interleukin-6 (IL-6) on tissue samples of DLBCL, NOS: IL-6–positive, with staining in the cytoplasm (A), and IL-6–negative (B) tissue samples (objective magnification, 40×).

Hb levels and platelet counts in patients with DLBCL, NOS, based on IL-6 expression in lymphoma cells. The mean Hb level was significantly higher in IL-6-negative cases than in

IL-6-positive cases (p = 0.0002) (C).

The mean platelet count in patients with bone marrow (BM) involvement was significantly lower than those without BM involvement (p = 0.0076) (D). In patients without BM involvement, the mean platelet count was significantly higher in IL-6-negative cases than in IL-6-positive cases (p = 0.0290) (E), and in patients with BM involvement the mean platelet count tended to be higher, not significantly, in IL-6-negative cases than in IL-6-positive cases (p = 0.2145) (F).

Table 1. Patients' characteristics

Characteristics		Validation cohort (n=94) Mean/ No. of patients (%)	Derivation cohort (n=66) Mean/ No. of patients (%)	Total Cohort (n=160) Mean/ No. of patients (%)
Age at diagnosis (years)	mean (range) ± SE	69.1 (28-92) ± 1.27	71.5 (32-98) ± 1.55	70.1 (28-98) ± 0.98
Sex	male/female	58 (62)/ 36 (38)	38 (58)/ 28 (42)	96 (60)/ 64 (40)
Clinical stage	I/II/III/IV	8 (9)/ 20 (21)/ 23 (24)/ 43 (46)	12 (18)/ 14 (21)/ 11 (17)/ 29 (44)	20 (13)/ 34 (21)/ 34 (21)/ 72 (45)
Bone marrow involvement	Present	25 (27)	21 (32)	46 (29)
	Absent	69 (73)	45 (68)	114 (71)
NCCN-IPI category	Low (L)	5 (5)	4 (6)	9 (5)
	Low-intermediate (LI)	25 (27)	18 (27)	43 (27)
	High-intermediate (HI)	36 (38)	31 (47)	67 (42)
	High (H)	28 (30)	13 (20)	41 (26)
Cell origin	GCB	41 (44)	19 (29)	60 (38)
	non-GCB	53 (56)	24 (36)	77 (48)
	unknown	0 (0)	23 (35)	23 (14)
Salvage therapy	R-MECP	11	10	21
	R-ESHAP	7	0	7
	R-GDP	4	0	4
	R-GCD	2	2	4
	Others	4	5	9
	R-Hyper-CVAD/MA	0	2	2
	R-EPOCH	0	1	1
	R-HD-MTX	1	1	2
	B-R	1	0	1
	R-VNCOP-B	1	0	1
	R-FD	1	0	1
	Radiation	0	1	1
Haemoglobin level (g/L)	mean (range) ± SE	119 (71-160) ± 2.16	119 (79-173) ± 2.43	119 (71-173) ± 1.61
male	≥ 130	20 (21)	11 (17)	31 (19)
	< 130	38 (41)	27 (41)	65 (41)
female	≥ 120	16 (17)	14 (21)	30 (18)
	< 120	20 (21)	14 (21)	34 (22)
White blood cell count ($\times 10^9$/L)	mean (range) ± SE	6.81 (2.30-18.3) ± 0.26	7.41 (2.90-26.1) ± 0.48	7.06 (2.30-26.1) ± 0.25
	≥ 4.0	85 (90)	61 (92)	146 (91)
	< 4.0	9 (10)	5 (8)	14 (9)
Platelet count ($\times 10^9$/L)	mean (range) ± SE	243 (11-625) ± 11.9	236 (26-496) ± 12.5	240 (11-625) ± 8.64
	≥ 100	84 (89)	63 (95)	147 (92)
	< 100	10 (11)	3 (5)	13 (8)
CRP level (mg/dL)	mean (range) ± SE	3.98 (0.009-27.8) ± 0.63	2.48 (0.01-12.3) ± 0.41	3.36 (0.009-27.8) ± 0.41
LDH level (U/L)	mean (range) ± SE	568 (137-7,043) ± 90.2	402 (148-1,251) ± 31.6	499 (137-7,043) ± 54.8
Albumin level (mg/dL)	mean (range) ± SE	3.49 (1.8-5.4) ± 0.09	3.82 (1.7-5.0) ± 0.09	3.62 (1.7-5.4) ± 0.06

Table 2. Relationship between haematologic parameters/ HPI risk groups and other clinical variables

	Leukocytopenia	Non-leukocytopenia	*p* value	Anaemia	Non-anaemia	*p* value	Thrombocytopenia	Non-thrombocytopenia	*p* value	HPI risk groups			*p* value
										Low	Intermediate	High	
No. (%)	14 (9)	146 (91)		99 (62)	61 (38)		13 (8)	147 (92)		60 (38)	88 (55)	12 (7)	
Sex (male/female)	7 (50)/7 (50)	89 (61)/57 (39)	0.424	65 (66)/34 (34)	31 (51)/30 (49)	0.0628	8 (62)/5 (38)	88 (60)/59 (40)	0.906	30 (50)/30 (50)	59 (67)/29 (33)	7 (58)/5 (42)	0.115
Age at diagnosis (years) mean ± SE	70.0 ± 3.34	70.1 ± 1.03	0.983	73.0 ± 1.20	65.3 ± 1.53	0.0001*	75.3 ± 3.44	69.6 ± 1.02	0.114	65.1 ± 1.54	72.8 ± 1.27	75.1 ± 3.44	0.0003*
Clinical stage													
I	3 (21)	17 (12)	–	7 (7)	13 (21)	–	0 (0)	20 (13)	–	13 (22)	7 (8)	0 (0)	–
II	1 (8)	33 (23)	–	19 (19)	15 (25)	–	2 (15)	32 (22)	–	14 (23)	19 (22)	1 (8)	–
III	3 (21)	31 (21)	–	25 (25)	9 (15)	–	2 (15)	32 (22)	–	9 (15)	23 (26)	2 (17)	–
VI	7 (50)	65 (44)	0.475	48 (49)	24 (39)	0.0256*	9 (70)	63 (43)	0.254	24 (40)	39 (44)	9 (75)	0.0429*
NCCN-IPI category													
Low	2 (14)	7 (5)	–	2 (2)	7 (11)	–	0 (0)	9 (6)	–	7 (12)	2 (2)	0 (0)	–
Low-intermediate	2 (14)	41 (28)	–	17 (17)	26 (43)	–	1 (8)	42 (28)	–	26 (43)	16 (18)	1 (8)	–
High-intermediate	4 (29)	63 (43)	–	48 (49)	19 (31)	–	3 (23)	64 (44)	–	18 (30)	47 (54)	2 (17)	–
High	6 (43)	35 (24)	0.146	32 (32)	9 (15)	< 0.0001*	9 (69)	32 (22)	0.0024*	9 (15)	23 (26)	9 (75)	< 0.0001*
Bone marrow involvement													
Present	6 (43)	40 (27)	–	33 (33)	13 (21)	–	8 (62)	38 (26)	–	13 (22)	25 (28)	8 (67)	–
Absent	8 (57)	106 (73)	0.222	66 (67)	48 (79)	0.103	5 (38)	109 (74)	0.0064*	47 (78)	63 (72)	4 (33)	0.0071*
Salvage therapy													
R-MECP	1 (25)	20 (49)	–	19 (53)	2 (22)	–	2 (67)	19 (45)	–	2 (22)	17 (52)	2 (67)	–
R-ESHAP	1 (25)	6 (14)	–	4 (11)	3 (34)	–	0 (0)	7 (17)	–	3 (34)	4 (12)	0 (0)	–
R-GDP	1 (25)	3 (7)	–	4 (11)	0 (0)	–	1 (33)	3 (7)	–	0 (0)	3 (9)	1 (33)	–
R-GCD	0 (0)	4 (10)	–	2 (6)	2 (22)	–	0 (0)	4 (10)	–	2 (22)	2 (6)	0 (0)	–
Others	1 (25)	8 (20)	0.658	7 (19)	2 (22)	0.135	0 (0)	9 (21)	0.424	2 (22)	7 (21)	0 (0)	0.268

*, statistically siginificant

Table 3. Significance of HPI risk groups and clinical variables as prognostic factors

Methods and Variables	Comparative factor /Reference factor	Relative risk for PFS (95% CI)	*p* value	Relative risk for OS (95% CI)	*p* value
Univariate analysis					
HPI	H/I	3.71 (1.84-7.47)	0.0003*	7.51 (3.46-16.3)	< 0.0001*
	H/L	10.2 (4.38-23.9)	< 0.0001*	21.3 (7.55-59.9)	< 0.0001*
	I/L	2.76 (1.44-5.27)	0.0022*	2.83 (1.15-7.00)	0.0242*
NCCN-IPI†	H/HI	2.72 (1.55-4.77)	0.0005*	5.80 (2.78-12.1)	< 0.0001*
	H/LI	5.39 (2.55-11.4)	< 0.0001*	9.09 (3.53-23.4)	< 0.0001*
	H/L	14.55 (1.95-109)	0.009*	–	–
Age (years)	> 60/ ≤ 60	1.61 (0.79-3.28)	1.61 (0.79-3.29)	1.61 (0.79-3.30)	1.61 (0.79-3.31)
Sex	M/F	1.38 (0.82-2.35)	0.228	1.59 (0.80-3.14)	0.182
Clinical stage	IV/II	3.03 (1.40-6.58)	0.005*	3.17 (1.20-8.37)	0.0202*
	IV/I	3.44 (1.22-9.75)	0.0198*	8.19 (1.10-60.8)	0.0399*
Bivariate analysis					
HPI	H/I	3.30 (1.61-6.73)	0.0011*	6.93 (2.99-16.0)	< 0.0001*
	H/L	6.76 (2.80-16.4)	< 0.0001*	13.9 (4.60-41.7)	< 0.0001*
	I/L	2.05 (1.04-4.05)	0.038*	–	–
NCCN-IPI†	H/HI	2.61 (1.47-4.64)	0.001*	5.79 (2.70-12.4)	< 0.0001*
	H/LI	4.04 (1.86-8.77)	0.0004*	7.07 (2.63-19.0)	0.0001*
	H/L	8.55 (1.10-66.3)	0.0401*	–	–

†Representative results with a significant p-value are shown among all combinations.

Table 4. Significance of haematopoietic parameters as prognostic factors

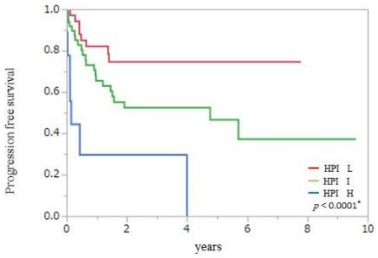
Methods and Variables	Relative risk for PFS (95% CI)	*p* value	Relative risk for OS (95% CI)	*p* value
Univariate analysis				
Leukocytopenia	1.11 (0.45-2.79)	0.817	1.19 (0.42-3.41)	0.743
Anaemia	3.37 (1.79-6.35)	0.0002*	4.03 (1.68-9.66)	0.0018*
Thrombocytopenia	3.94 (1.99-7.80)	< 0.0001*	7.56 (3.63-15.75)	< 0.0001*
Multivariate analysis				
Anaemia	3.11 (1.64-5.89)	0.0005*	3.51 (1.45-8.50)	0.0053*
Thrombocytopenia	3.23 (1.62-6.42)	0.0008*	6.38 (3.03-13.45)	< 0.0001*

Table 5. Histological features and HPI risk groups based on IL-6 expression in lymphoma cells

Valuables (number of analized cases)	Mean (range) /No. of patients (%)		*p* value
	IL-6 positive cases	IL-6 negative cases	
Ki-67 index (n=102)	66.9 (62.1-71.8) ± 2.45/ 71(70)	65.8 (58.5-73.2) ± 3.70/ 31(30)	0.806
Cell origin (n=108)			
GCB type (n=45)	23 (31)	22 (65)	–
Non-GCB type (n=63)	51 (69)	12 (35)	0.001*
MYC expression (n=96)			
positive (n=31)	22 (33)	9 (31)	–
negative (n=65)	45 (67)	20 (69)	0.862
Biologic phenotype (n=96)			
Double expressor (n=24)	16 (24)	8 (28)	–
Non-double expressor (n=72)	51 (76)	21 (72)	0.700
HPI risk groups (n=111)			
Low (n=40)	22 (29)	18 (50)	–
Intermidiate (n=61)	45 (60)	16 (44)	
High (n=10)	8 (11)	2 (6)	0.097

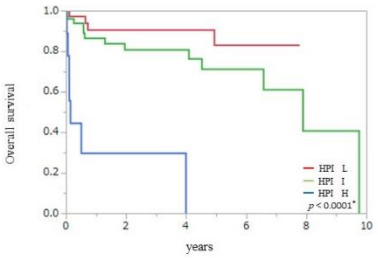
FIGURE 1

(A)



Number at risk						
L	35	17	11	5	1	1
LI	50	20	12	3	2	1
H	9	2	1	1	1	1

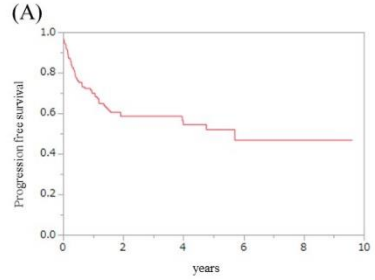
(B)



Number at risk						
L	35	22	14	6	2	2
LI	50	28	19	9	3	1
H	9	2	1	1	1	1

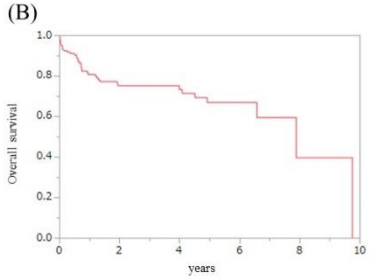
FIGURE 2

(A)



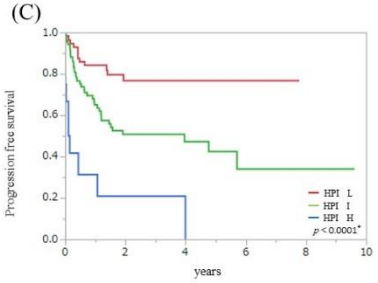
Number at risk						
All	160	59	28	7	2	1

(B)



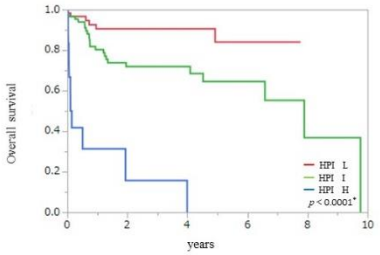
Number at risk						
All	160	75	40	14	3	1

(C)



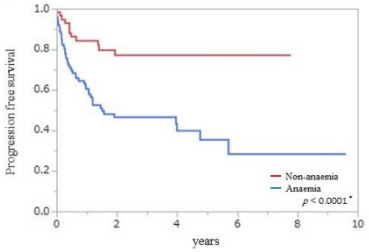
Number at risk						
L	60	30	15	5	1	1
LI	88	29	14	3	2	1
H	12	2	1	1	1	1

(D)



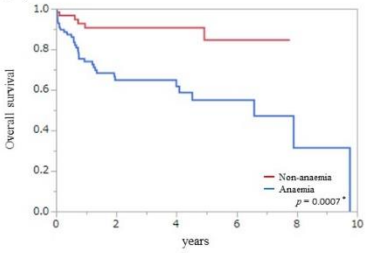
Number at risk						
L	60	36	19	6	2	2
LI	88	39	22	9	3	1
H	12	2	1	1	1	1

(E)



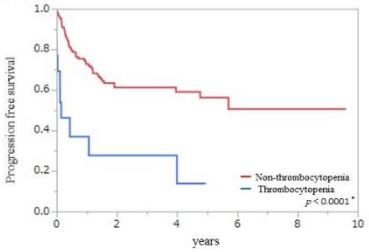
Number at risk						
Non-anaemia	61	31	16	5	1	1
Anaemia	99	29	13	3	2	1

(F)



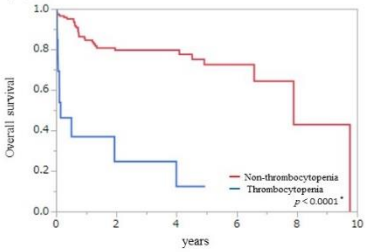
Number at risk						
Non-anaemia	61	37	20	6	2	2
Anaemia	99	39	21	9	3	1

(G)



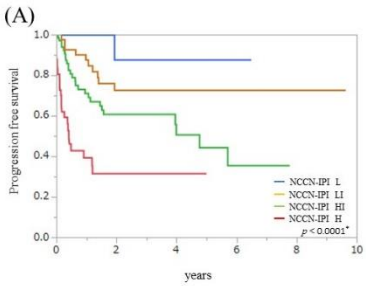
Number at risk						
Non-thrombocytopenia	147	57	27	7	2	1
Thrombocytopenia	13	3	2	1	1	1

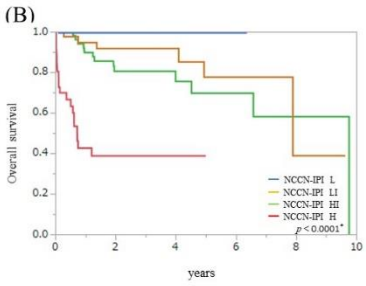
(H)



Number at risk						
Non-thrombocytopenia	147	73	39	14	3	1
Thrombocytopenia	13	3	2	1	1	1

FIGURE 3

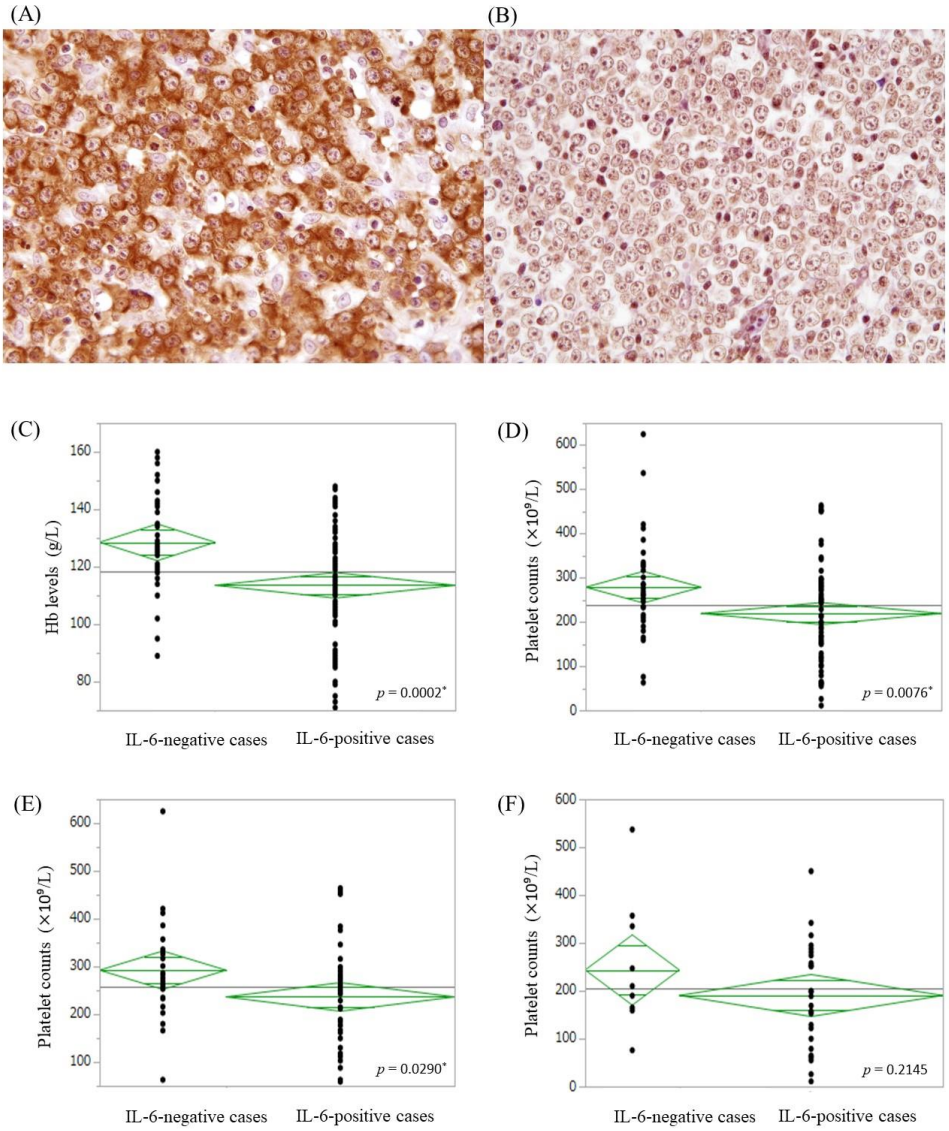




Number at risk						
L	9	8	6	3	1	1
LI	43	22	11	3	2	1
HI	67	26	11	3	1	1
H	41	6	3	1	1	1

Number at risk						
L	9	9	8	3	1	1
LI	43	28	15	6	2	1
HI	67	33	16	7	2	1
H	41	8	4	1	1	1

FIGURE 4


(A)
(B)
(C)
Hb levels (g/L)
160
140
120
100
80
p = 0.0002*
IL-6-negative cases
IL-6-positive cases
(D)
Platelet counts (×10⁹/L)
600
500
400
300
200
100
0
p = 0.0076*
IL-6-negative cases
IL-6-positive cases
(E)
Platelet counts (×10⁹/L)
600
500
400
300
200
100
p = 0.0290*
IL-6-negative cases
IL-6-positive cases
(F)
Platelet counts (×10⁹/L)
600
500
400
300
200
100
0
p = 0.2145
IL-6-negative cases
IL-6-positive cases