bjh research paper

New prognosis score including absolute lymphocyte/monocyte ratio, red blood cell distribution width and beta-2 microglobulin in patients with diffuse large B-cell lymphoma treated with R-CHOP: Spanish Lymphoma Group Experience (GELTAMO)

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Summary

The International Prognostic Index (IPI) is the most widely used score for non-Hodgkin lymphoma but lacks the ability to identify a high-risk population in diffuse large B-cell lymphoma (DLBCL). Low absolute lymphocyte count and high monocytes have proved to be unfavourable factors. Red-cell distribution width (RDW) has been associated with inflammation and beta-2 microglobulin (B2M) with tumour load. The retrospective study included 992 patients with DLBCL treated with R-CHOP. In the multivariate analysis, age, Eastern Cooperative Oncology Group performance status (ECOG-PS), stage, bulky mass, B2M, RDW, and lymphocyte/monocyte ratio (LMR) were independently related to progression-free survival (PFS). A new prognosis score was generated with these variables including age categorized into three groups (0, 1, 2 points); ECOG $\geq$ 3–4 with two; stage III/IV, bulky mass, high B2M, LMR $<$ 2·25 and RDW $>$ 0·96 with one each; for a maximum of 9. This score could improve the discrimination of a very high-risk subgroup with five-year PFS and overall survival (OS) of 19% and 24% *versus* 45% and 59% of R (revised)-IPI respectively. This score also showed greater predictive ability than IPI. A new score is presented including complete blood cell count variables and B2M, which are readily available in real-life practice without additional tests. Compared to R-IPI, it shows a more precise high-risk assessment and risk discrimination for both PFS and OS.

Keywords: diffuse large B-cell lymphoma, prognosis, complete blood cell counts, β2-microglobulin, index.

First published online 28 November 2019
doi: 10.1111/bjh.16263

Received 2 September 2019; accepted for publication 30 September 2019
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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of lymphoid neoplasm characterized as a heterogeneous group of aggressive lymphomas. Treatment of DLBCL is relatively homogeneous and standard, mainly based on the R-CHOP regimen [rituximab, cyclophosphamide, hydroxydaunorubicin hydrochloride (doxorubicin hydrochloride), vincristine (Oncovin) and prednisone] that produces complete remission (CR) rates of around 70–90% (Coiffier *et al*., 2002; Pfreundschuh *et al*., 2006), and five-year progression-free survival (PFS) and overall survival (OS) of around 60–70% (Feugier *et al*., 2005). However, 30–40% of patients still fail this standard therapy, so efforts to improve outcomes by new approaches, adding new drugs or novel biological agents, are needed. For this purpose, the most critical point is how to identify patients at high risk of failure with standard therapy.

Several prognostic scores have been proposed for categorizing the risk and finding those with worse results with standard treatment. The most important and widely used is the International Prognostic Index (IPI) proposed in 1993 (A predictive model for aggressive non-Hodgkin lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project, 1993; Zhou *et al*., 2014) and lately validated in the rituximab era (R-IPI) (Sehn *et al*., 2007). Five binary clinical variables including age, lactate dehydrogenase (LDH), number of extranodal sites, Ann Arbor stage and Eastern Cooperative Oncology Group performance status (ECOG-PS) were used to stratify and identify four discrete risk categories that were reduced to three in the R-IPI. However, despite being a good prognostic score, it lacks the ability to identify a very high-risk prognostic subset in the rituximab era: the high-risk group of R-IPI has four-year OS and PFS greater than 50%. In the last few years, several attempts have been made to try to improve the discrimination ability of IPI, such as National Cancer Comprehensive Network (NCCN)-IPI, but mainly without including new prognostic factors.

Likewise, new predictors of prognosis involving molecular and cytogenetic, as well as other biological and clinical markers have been proposed. However, to improve current prognostic scores in DLBCL, the inclusion of very sophisticated new biomarkers — which might be challenging to obtain rapidly in real -life — must be balanced with new, informative variables that are widely and quickly available and as such would be able to help in real-life risk assessment. Among these readily available clinical markers, complete blood cell counts (CBC) have been investigated due to their prognostic value in the treatment of DLBCL, since the tests are simple to perform, inexpensive and easily accessible (Chen *et al*., 2016) and also as there is increasing evidence that tumour microenvironment and host immunity play an important role in lymphoma progression.

This study aimed to develop a readily available 'user-friendly' prognostic score using easy to obtain variables, evaluating the discriminative ability of the new score in comparison with previously reported ones.

Materials and methods

Patients and sample selection

The files of 2156 patients with DLBCL were retrieved from the archives of 20 academic and community hospitals in the GELTAMO network in Spain that was used for the validation of NCCN-IPI and development of the GELTAMO-IPI, as previously described (Montalbán *et al*., 2017). Patients were diagnosed between January 1998 and July 2014. For the present study, cases that received frontline induction therapy with R-CHOP with or without radiotherapy, with a minimum follow-up of one year and with the easy to obtain experimental variables available (CBC data and B2M) were selected from the original GELTAMO DLBCL database (n = 992). Treatments with non-curative intent were excluded. This study was approved by the Ethics Committee (EC) of the Hospital Ramon y Cajal (Madrid, Spain), which is the reference EC.

Patients were only excluded on the grounds of unavailability of data, which were missing randomly and independently of outcomes, so there was no intentional bias in the selection. A diagnosis of DLBCL was established upon the initial lymph node biopsy or a biopsy from the primary extranodal site by local pathologists in the contributing centres. All histological subtypes of DLBCL and patients positive for hepatitis B virus, hepatitis C virus and human immunodeficiency

virus were also eligible. Primary testicular and central nervous-system lymphomas were not.

Baseline clinical characteristics, recorded at the time of diagnosis, were age, gender, Ann Arbor stage, LDH and B2M serum levels, B symptoms, bulky disease (>10 cm) and ECOG-PS. Additionally, CBC variables were recorded [absolute lymphocytes (ALC), monocyte and neutrophil count, RDW, platelets and haemoglobin]. All quantitative variables were standardized using the normal reference values of each centre.

Complete remission was accepted after the resolution of all lymph nodal and extranodal involvement based on image techniques [computed tomography (CT) in all cases, although positron emission tomography may have been used in some patients] and, when available, on a biopsy of previously affected extranodal areas.

Statistical methods

The primary endpoint was PFS, defined as the time from diagnosis to refractoriness (lack of CR at the end of induction or early progression), relapse or death from any cause. As an evaluation of CR may differ between the participating hospitals or the period times, including Cheson or Lugano criteria, cases with less than 12 months follow-up were excluded to avoid sensitivity or specificity bias related to different response criteria in terms of progression identification. OS was calculated from the date of diagnosis until death from any cause. PFS and OS were analysed with the Kaplan–Meier method and compared using the log-rank test. Cox regression models were used for univariate and multivariate analysis.

Development of the new score

To develop the new score, the series was randomly split into training and validation cohorts. For this purpose, we used the standard spss (IBM, New York, NY, USA) randomization system in a proportion of approximately 80% and 20%, respectively. The training set was used to derive the model through univariate and multivariate Cox regression PFS models. Selection of the new variables to be included in the new score took into account the CBC values standardized using the reference values of each centre. Optimal cutoffs of quantitative variables were calculated from the training set through Maxstat (maxstat package for R software environment), using restricted cubic splines and minimizing Martingale residuals when more than one cutoff may be necessary. Comparisons between scores were performed using the C-index.

Results

Clinical features and univariate analysis

A total of 992 patients were retrospectively analysed. Presenting features of the patients are shown in Table I. Median age at diagnosis was 64 years (range, 18–91 years) and median follow-up time was 55 months (range, 12–185). For all patients, the overall response rate was 90% and the CR rate was 79%. The five-year PFS and OS were 61% and 74%, respectively. Survival analysis with standard clinical prognostic factors and new experimental variables is shown in Table II. The ALC, absolute monocyte count (AMC), LMR, RDW, B2M and the rest of the characteristics analysed were significantly associated with PFS.

Development of the new prognostic model

After splitting the series into training and validation cohorts, two sets of patients were obtained with mostly similar clinical characteristics as shown in Table I. However, the training cohort had significantly more males and more patients with elevated B2M.

The initial optimal cut-off points of the quantitative variables were determined by the Maxstat method: ALC 1·1, AMC 0·79, LMR 2·25, RDW 0·96, haemoglobin 1·01, LDH ratio 1 and 1·96, age 60 and 75 and B2M 1·04 (Fig 1).

In the multivariate analysis, age, poor ECOG-PS, Ann Arbor III/IV stage, bulky mass, high B2M, LMR < 2·25 and RDW > 0·96 were independent predictors of shorter PFS. These seven independently significant features were used to design a new model to predict an individual patient's PFS. The model was scored considering the hazard ratio of each category of the variables, which was included as follows (Table III): age, 0, 1 and 2 points for three different levels; ECOG-PS, 0 and 2 points; Ann Arbor III/IV stage, bulky mass, high B2M, LMR < 2·25 and RDW > 0·96, 0 or 1 point each. Scores for the individual factors were added to arrive at a total score ranging from 0 to 9. Four risk groups were formed on the basis of the shape of the Kaplan–Meier curves for PFS: low risk (0 points), low intermediate (1–3), high intermediate (4–6) and high risk (7–9). This score was validated using the validation cohort (Fig 2).

Comparison of the new prognostic model with previously reported scores

The new score showed better discrimination of outcomes than the IPI for both PFS and OS (Fig 3). With the new model, the highest-risk group had five-year PFS of 19% and five-year OS of 24%, favourable compared with IPI (45% and 59%, respectively). Likewise, a better high-risk assessment of the new score was observed when compared to previously reported scores such as Tumor Score (TS), NCCN-IPI and GELTAMO-IPI (Rodriguez *et al.*, 1992; Zhou *et al.*, 2014; Montalbán *et al.*, 2017), which showed higher five-year PFS and five-year OS (32%, 38%, 29% and 45%, 46%, 40% respectively).

The new score also showed greater predictive ability than the IPI for PFS (C-index, 0·69 for the new model vs. 0·65 for the IPI, $P = 0·002$) and OS (C-index, 0·73 for the new model vs. 0·67 for the IPI, $P = 0·018$).

Table I. Patient characteristics.

	Global group (n = 992)	Training sample (n = 780)	Validation sample (n = 212)	P
Median age (range)	64 (18–91)	64 (18–91)	64 (18–85)	1
Gender M/F (%)	498 (50%)/489 (50%)	406 (52%)/370 (47%)	92 (43%)/119 (56%)	0·03
ECOG-PS >1	287 (30%)	232 (30%)	55 (26%)	0·27
Stage III/IV	604 (61%)	480 (62%)	124 (58%)	0·38
B symptoms	383 (39%)	305 (39%)	78 (37%)	0·63
High LDH	556 (58%)	448 (60%)	108 (53%)	0·11
>1 extranodal site	179 (18%)	135 (17%)	44 (21%)	0·27
Bulky mass	297 (32%)	232 (31%)	65 (33%)	0·67
High beta-2-microglobulin	440 (49%)	363 (52%)	77 (39%)	0·003

M, male; F, female; ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase.

Table II. Survival analysis with standard clinical prognostic factors and new experimental variables in the training sample.

	PFS		OS	
	HR (95% CI)	P	HR (95% CI)	P
Age				
0–60	–	<0·001	–	<0·001
>60–75	1·57 (1·21–2·04)	0·001	1·85 (1·32–2·6)	<0·001
>75	2·44 (1·8–3·3)	<0·001	3·55 (2·47–5·08)	<0·001
Male gender	1·25 (1–1·57)	0·049	1·48 (1·12–1·95)	0·006
ECOG-PS				
0–1	–	<0·001	–	<0·001
2	1·63 (1·26–2·12)	<0·001	2·02 (1·47–2·78)	<0·001
3–4	3·13 (2·26–4·35)	<0·001	4·23 (2·91–6·15)	<0·001
Stage III–IV	2·08 (1·62–2·68)	<0·001	2·33 (1·7–3·2)	<0·001
B-symptoms	2·05 (1·64–2·55)	<0·001	2·42 (1·84–3·19)	<0·001
LDH ratio				
0–1	–	<0·001	–	<0·001
>1–1·96	1·66 (1·27–2·19)	<0·001	1·62 (1·15–2·28)	0·005
>1·96	2·37 (1·78–3·16)	<0·001	2·47 (1·74–3·51)	<0·001
>1 extranodal site	1·76 (1·36–2·29)	<0·001	1·59 (1·14–2·21)	0·006
Bulky disease	1·5 (1·19–1·90)	0·001	2·42 (1·84–3·19)	<0·001
High Beta-2-microglobulin	2·19 (1·7–2·8)	<0·001	3·17 (2·29–4·4)	<0·001
LMR 0–2·25	1·87 (1·49–2·34)	<0·001	1·54 (1·17–2·03)	0·002
Lymphocytes <1·1	1·62 (1·29–2·02)	<0·001	1·53 (1·16–2·01)	0·003
Monocytes >0·79	1·66 (1·32–2·07)	<0·001	1·56 (1·19–2·06)	0·001
RDW ratio >0·96	1·84 (1·47–2·31)	<0·001	1·85 (1·4–2·44)	<0·001
Hemoglobin below normal limit	1·53 (1·22–1·92)	<0·001	1·83 (1·37–2·43)	<0·001

ECOG, Eastern Cooperative Oncology Group; PS, performance status; LDH, lactate dehydrogenase; LMR, lymphocyte/monocyte ratio; RDW, red blood cell distribution width.

Discussion

Several prognostic scores have been proposed for categorizing risk and finding DLBCL with worse results with standard treatment, suitable to be treated with new schemes or drugs. The NCCN-IPI, compared with the IPI, incorporates age and LDH, achieving an enhanced capacity to discriminate low- and high-risk groups (Zhou *et al*., 2014; El-Galaly *et al*., 2015). With the same aim, our group recently reported the GELTAMO-IPI, including all variables of IPI, with a further categorization of age and ECOG-PS, as well as B2M (Montalbán *et al*., 2017). In 1992, the MD Anderson Cancer Centre reported the TS exclusively considering variables related to the tumour: high LDH, Ann Arbor stage III/IV, high B2M, bulky mass and the presence of B symptoms (Rodriguez *et al*., 1992). This score has recently been validated by our group in the rituximab era, improving the ability to identify high-risk

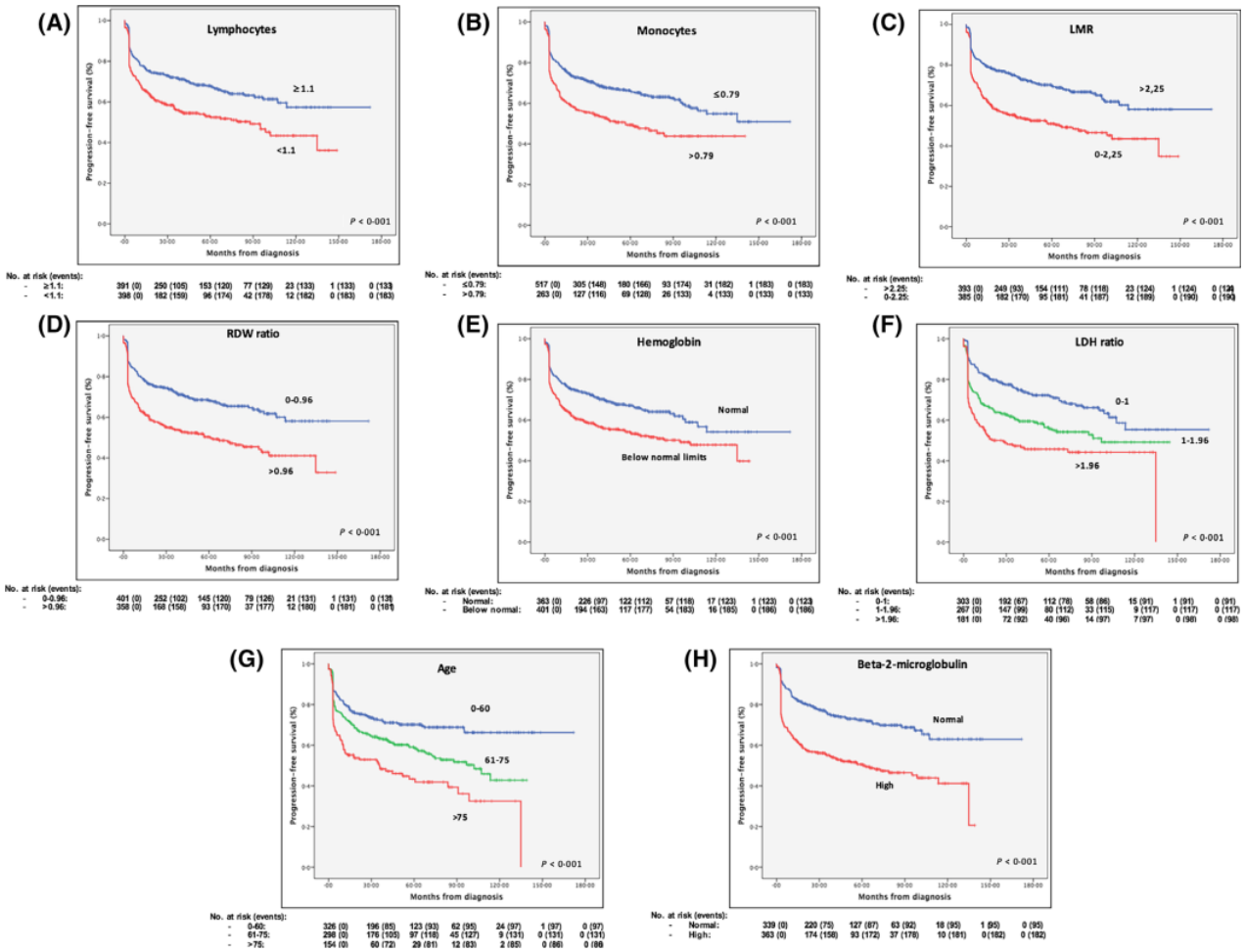

Fig 1. Cutoff of quantitative variables (A–H) from the training sample.

Table III. Score development considering independent variables of multivariate analysis for PFS in the training sample.

New score	HR (95% CI)	*P*	Points
Age			
<60	–	–	0
60–75	1·36 (1·01–1·83)	0·045	1
>75	2·09 (1·51–2·9)	<0·001	2
ECOG-PS			
0–2	–	–	0
3–4	2·34 (1·63–3·38)	<0·001	2
Stage III/IV	1·48 (1·1–2)	0·011	1
Bulky mass	1·55 (1·2–2)	0·001	1
High B2M	1·44 (1·1–1·9)	0·009	1
LMR 0–2·25	1·39 (1·07–1·8)	0·013	1
RDW ratio >0·96	1·39 (1·07–1·8)	0·014	1

ECOG, Eastern Cooperative Oncology Group; PS, performance status; B2M, beta-2-microglobulin; LMR, lymphocyte/monocyte ratio; RDW, red blood cell distribution width.

subsets (Gutiérrez *et al.*, 2017). However, even better discrimination of the high-risk group for both PFS and OS is needed.

The prognostic significance of CBC parameters has been well established in some solid tumours (Teramukai *et al.*, 2009). In the context of lymphoma, there is increasing evidence that tumour microenvironment and host immunity play an important role in progression (Lenz *et al.*, 2008). Many retrospective studies have assessed the role of ALC at diagnosis in predicting outcomes of DLBCL (Cox *et al.*, 2008b; Chae *et al.*, 2012) and have reported that a higher ALC at the time of diagnosis is associated with improved PFS and OS, independently of the IPI. Attempts have been made to devise a new prognostic scoring system that incorporates the ALC at diagnosis with the IPI. Cox *et al.* described a new trichotomous score (ALC/R-IPI) that classified patients into low risk (R-IPI very good or good and ALC $\geq 0{\cdot}84 \times 10^9$/l), intermediate risk (R-IPI poor or ALC $\geq 0{\cdot}84 \times 10^9$/l) and high risk (R-IPI poor and ALC $< 0{\cdot}84 \times 10^9$/l) (Cox *et al.*, 2008a). On multivariate analysis, this ALC/R-IPI score was the most powerful predictor in their cohort for PFS and OS.

Moreover, the number of monocytes detectable in peripheral blood has been investigated, with the results indicating this may possibly affect the pathogenesis and prognosis of

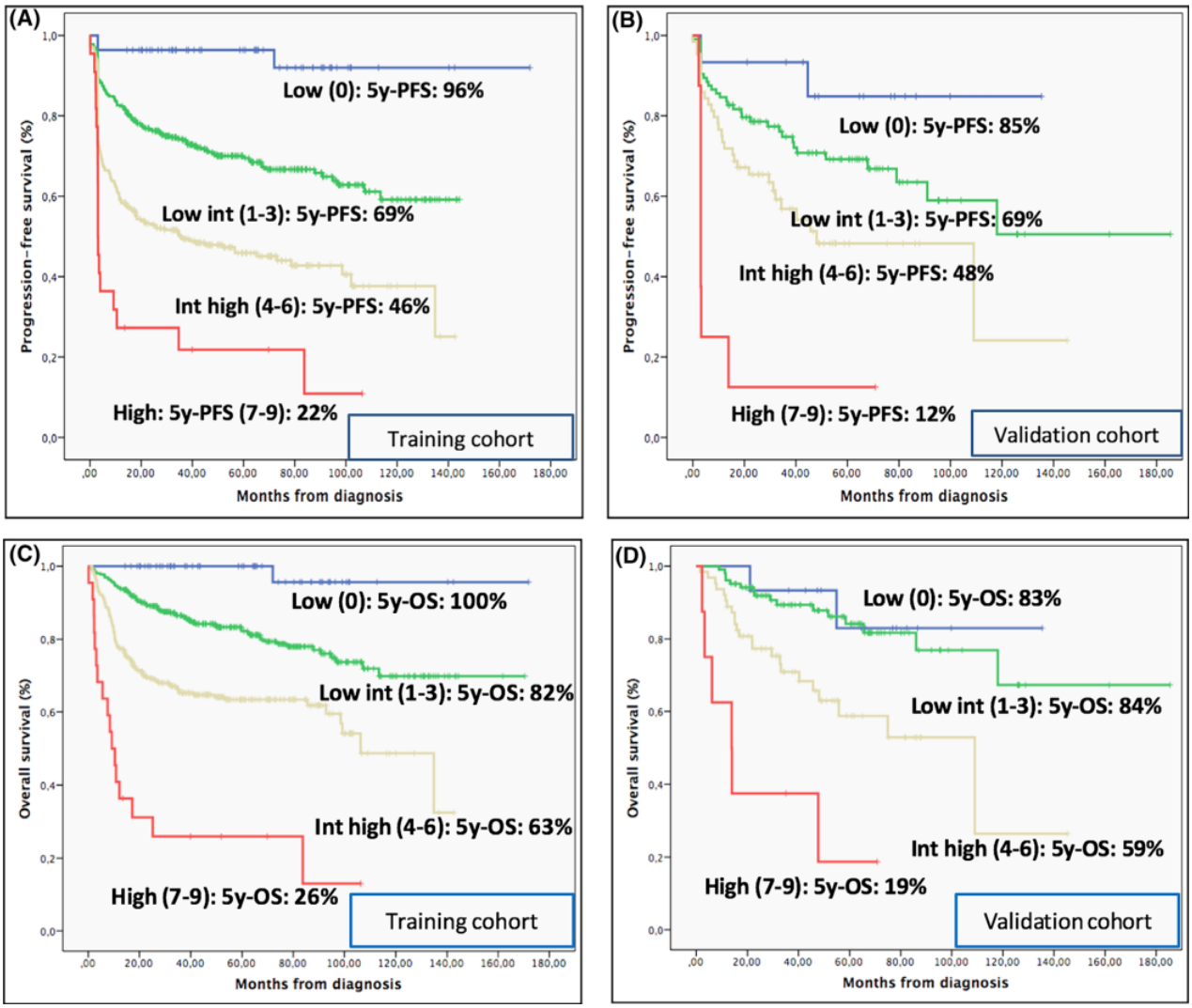

Fig 2. Score validation. (A) Progression-free survival (PFS) in training cohort; (B) PFS in validation cohort; (C) Overall survival (OS) in training cohort; (D) OS in validation cohort.

this disease (Mueller *et al.*, 2007). In contrast to ALC, high levels of blood monocytes have just the opposite effect. Furthermore, the lymphocyte to monocyte ratio (LMR) assessed at diagnosis may predict the clinical outcome of DLBCL patients (Rambaldi *et al.*, 2013; Porrata *et al.*, 2014). Wilcox *et al.* described a dichotomous absolute monocyte count (AMC)/ALC prognostic score that stratified patients into three risk groups: low risk (AMC $<$ 630/ml and ALC $>$ 1000/ml), intermediate risk (AMC $\geq$ 630/ml or ALC $\leq$ 1000/ml) and high risk (AMC $\geq$ 630/ml and ALC $\leq$ 1000/ml) (Wilcox *et al.*, 2011). Li *et al.* used an inverse LMR at diagnosis in their study with similar results (Li *et al.*, 2012). We confirmed that LMR predicted both shorter PFS and OS in our series.

Peripheral platelet counts and haemoglobin levels have also been reported to be prognostic determinants for DLBCL (Troppan *et al.*, 2015; Yamauchi *et al.*, 2015). In our study, PFS and OS were not influenced by platelet count and haemoglobin levels did not independently influence survival. Red blood cell distribution width (RDW) is an easy-to-measure marker of the systemic inflammatory response that is involved in many pathophysiological conditions, including cardiovascular disease and generally increased progressive inflammation (Förhécz *et al.*, 2009; Lippi *et al.*, 2009) and has traditionally played a role in the differential diagnosis of anaemia (Weiss & Goodnough, 2005). Recently, RDW is being increasingly recognized as playing a significant role in carcinogenesis and tumour progression (Koma *et al.*, 2013). Although the mechanism underlying the relationship between RDW and survival or disease activity is not fully understood, it has been associated with a variety of inflammatory markers, such as high-sensitivity C-reactive protein, erythrocyte sedimentation rate, interleukin-6, soluble transferrin receptor and soluble tumour necrosis factor receptors I and II (Lippi *et al.*, 2009). The role of inflammation in the development of lymphoma has long been recognized and investigated extensively. DLBCL development and invasion depend on multiple

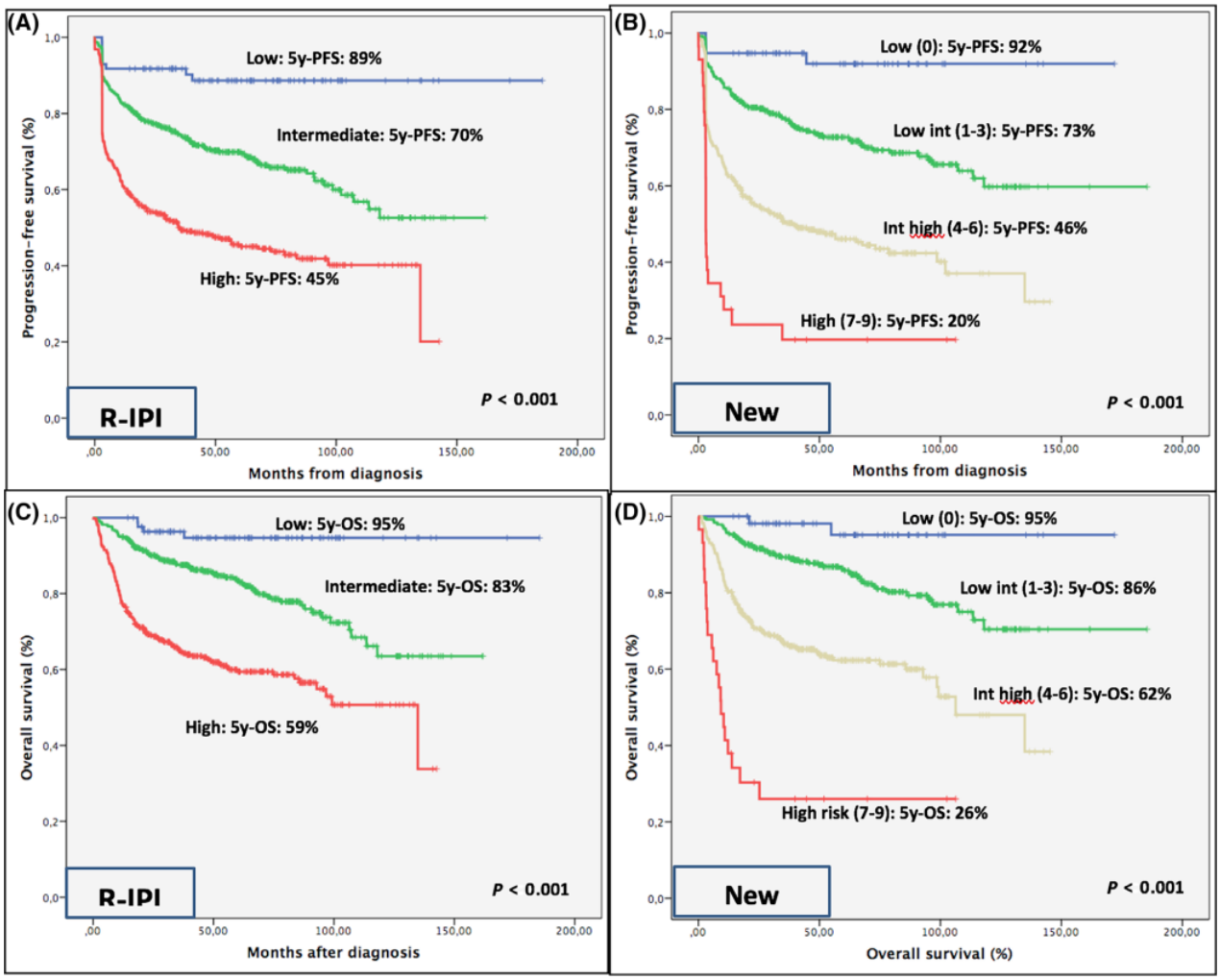

Fig 3. Comparison of the new prognostic model with the revised International Prognostic Index (R-IPI). (A) Progression-free survival (PFS) depending on R-IPI; (B) PFS depending on new score. (C) Overall survival (OS) depending on R-IPI; (D) OS depending on new score.

interactions between tumour and non-neoplastic cells and their interaction with the surrounding stroma/matrix environment (Mbeunkui & Johann, 2009). Some studies have confirmed that high RDW obtained at diagnosis as part of a standard automated CBC predicted an unfavourable prognosis in patients with DLBCL (Zhou *et al*., 2017). In our study, RDW was an independent variable for PFS and OS.

Beta-2 microglobulin is a small polypeptide light chain that forms part of the major histocompatibility complex class I antigens. Its role as an adverse prognostic factor in lymphoproliferative diseases (lymphoma and myeloma) is not fully understood. Several studies have shown its prognostic role in DLBCL in both the pre- (Rodriguez *et al*., 1992) postrituximab eras (Miyashita *et al*., 2015). As white-blood-cell membrane turnover is the primary source of serum B2M, elevated serum B2M levels have been associated with lymphoid malignancies with significant tumour burden, high rates of cellular turnover and more invasive potential. Since B2M is mainly excreted by the kidneys, renal failure might be a cause of serum elevation as well as in situations of increased systemic or local inflammation associated with, for example, cardiovascular disease or aging (Shinkai *et al*., 2008). Hence, increased serum B2M levels may be an adverse prognostic factor, both tumour- and/or host related. The addition of B2M to the main variables of IPI clearly improves risk assessment in DLBCL as was recently reported in the GELTAMO-IPI (Montalbán *et al*., 2017) and has recently been confirmed in an independent series (Hong *et al*., 2017).

In our study, a score including LMR, RDW and B2M was analysed for the first time together with other clinical variables. LMR, RDW and B2M were demonstrated to predict shorter PFS. A new prognostic model was then constructed based on LMR, RDW and B2M in combination with age, ECOG-PS, stage and bulky mass, showing significantly better risk discrimination than IPI and ability to identify a high-risk group with five-year PFS of 19% and five-year OS of 24%.

In the last few years, some cytogenetic markers have demonstrated an impact on prognosis such as translocations involving

the MYC oncogene (present in 5–10% of DLBCL) (Klapper *et al.*, 2008). The adverse prognostic effect of MYC translocation seems to increase in the presence of an additional chromosomal breakpoint involving the BCL2 or BCL6 loci. These '*double-hit*' lymphomas seem to have an extremely aggressive clinical course and poor response to standard chemotherapy (Sha *et al.*, 2019). Besides, gene expression profiling for the cell of origin is a genomics tool that utilizes DNA microarray to assess gene expression and at least three molecularly distinct forms of DLBCL have been identified: the germinal centre B-cell, activated B-cell and primary mediastinal B-cell subtypes (Alizadeh *et al.*, 2000). Unfortunately, while immensely powerful, it is not applicable to routine clinical practice; and other approaches such as immune-histochemical algorithms to translate the robust molecular information into routine clinical use are controversial (Gutiérrez-García *et al.*, 2011).

A new prognosis score including CBC variables (LMR and RDW) and B2M is presented for the first time, which are readily available in real-life practice without additional tests. Compared to R-IPI, this score showed better high-risk assessment for both PFS and OS. Furthermore, better risk discrimination for PFS and OS was confirmed with the new score compared to IPI. Prospective trials with new approaches or drugs are needed to improve the outcomes of the very high-risk subgroup identified.

Declaration statements

Ethic approval and consent to participate: The study was approved by the Ethics Committee of the Hospital Ramón y Cajal (Madrid, Spain).

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

This study was supported by grants from the Plan Nacional de I+D+I, co-financed by the ISCIII-Subdirección General de Evaluación, Fondo Europeo de Desarrollo Regional (FEDER), PI12/1832, B2017/BMD-3778 from the Comunidad de Madrid and project SYN17/04 from IdISBa.

Authors' contributions

LB and AG performed the research. LB, ADL, GB, AMMM, MB, AM, JMS, OG, MR, JMSP, SN, AS, MB, MJRS, SGDV, RC, MGR, JMS, RC, HL, DG, AH, PA, JR and AG contributed clinical data. LB and AG contributed to the analysis and data interpretation. AG, JSS and PR served as the statisticians. All authors contributed to the review, provided their comments on this manuscript and approved the final version.

Conflict of interest

LB reports honoraria as speaker or advisory board member from Janssen, Amgen and Roche. AM discloses consultancy to Servier, Gilead and Celgene. AG discloses consultancy and research funding from Roche and Janssen. The other authors have no conflicts of interest to declare.

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