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Research Article

Generation and External Validation of a Histologic Transformation Risk Model for Patients with Follicular Lymphoma

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ABSTRACT

Follicular lymphoma (FL) is the most frequent indolent lymphoma. Some patients (10%-15%) experience histologic transformation (HT) to a more aggressive lymphoma, usually diffuse large B-cell lymphoma (DLBCL). This study aimed to validate and improve a genetic risk model to predict HT at diagnosis.We collected mutational data from diagnosis biopsies of 64 FL patients. We combined them with the data from a previously published cohort (total n = 104; 62 from nontransformed and 42 from patients who did transform to DLBCL). This combined cohort was used to develop a nomogram to estimate the risk of HT. Prognostic mutated genes and clinical variables were assessed using Cox regression analysis to generate a risk model. The model was internally validated by bootstrapping and externally validated in an independent cohort. Its performance was evaluated using a concordance index and a calibration curve.

The clinicogenetic nomogram included the mutational status of 3 genes (*HIST1HE1*, *KMT2D*, and *TNFSR14*) and high-risk Follicular Lymphoma International Prognostic Index and predicted HT with a concordance index of 0.746. Patients were classified as being at low or high risk of transformation. The probability HT function at 24 months was 0.90 in the low-risk group vs 0.51 in the high-risk

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group and, at 60 months, 0.71 vs 0.15, respectively. In the external validation cohort, the probability HT function in the low-risk group was 0.86 vs 0.54 in the high-risk group at 24 months, and 0.71 vs 0.32 at 60 months. The concordance index in the external cohort was 0.552. In conclusion, we propose a clinicogenetic risk model to predict FL HT to DLBLC, combining genetic alterations in *HIST1H1E*, *KMT2D*, and *TNFRSF14* genes and clinical features (Follicular Lymphoma International Prognostic Index) at diagnosis. This model could improve the management of FL patients and allow treatment strategies that would prevent or delay transformation.

Introduction

Follicular lymphoma (FL) is the second most common type of B-cell non-Hodgkin lymphoma, with an annual incidence of 3 to 5 per 100,000 habitants in the United States and Europe.[1] FL, a heterogenous but generally indolent disease, is characterized by slow progression, high response rates to therapy, and long overall survival (OS). However, it remains an incurable disease.[2] Approximately 20% of patients experience disease progression within 24 months of treatment (POD24) and face poor outcomes.[2,3] Additionally, 10% to 30% of patients experience transformation to high-grade lymphoma, most frequently to a diffuse large B-cell lymphoma (DLBCL).[4-6] The histologic transformation (HT) event, which occurs in 2% to 3% of patients annually, has been associated with treatment resistance and worse prognosis and is a critical process in FL patients.[4,7-10] It is still challenging to predict transformation at diagnosis. Several studies have associated clinical risk factors, such as the Follicular Lymphoma International Prognostic Index (FLIPI), advanced stage, Eastern Cooperative Oncology Group (ECOG) performance status, and histologic grade, with a higher risk of transformation, but none of these is specific to HT.[6,11,12] An association between early progression and risk of transformation has also been described,[13] both events associated with shorter OS. However, HT may occur in patients without evidence of progression and vice versa: some patients progress without further transformation.

From a molecular point of view, the transformed DLBLC sample (tFL) is more complex genomically than the precedent FL sample (pre-tFL). Pre-tFL samples are more complex than nontransformed (ntFL) ones.[14-16] HT has previously been associated with alterations in *TP53*, *CDKN2A*, *MYC*, *CCND3*, *GNA13*, and SOCS1, among other genes.[14,15,17,18] A recent study by Dreval et al[19] shed some additional light on the FL transformation process and, using whole-genome sequencing, described 2 genetically distinct subgroups of FL, DLBCL-like (dFL) and constrained FL (cFL), whose time to transformation differed significantly. Each subtype had different mutational landscapes and aberrant somatic hypermutation rates. The cFL subtype was associated with a lower mutational burden, in line with our previous results.[15] The dFL subtype showed a similar mutational pattern to that in DLBCL, including, for example, a higher incidence of *GNA13* mutations. Our results and others revealed greater genomic complexity in pre-tFL than in ntFL samples, with higher rates of subclonal mutations.[15,17,18] The transformation process is the consequence of genetic alterations in tumor B cells and the result of interactions between lymphoma cells and the immune system. The type and distribution of immune cells that infiltrate the tumor microenvironment have been proposed as predictors of transformation.[20]

In 2019, González-Rincón et al[15] published a genetic model to predict HT at diagnosis in patients with FL based on mutations in the *NOTCH2*, *DTX1*, *UBE2A*, and *HIST1H1E* genes. The multivariate Cox regression coefficients classified patients into low-, intermediate-, and high-risk groups. In the present study, we have generated and validated an improved clinicogenetic model to predict the HT of FL into DLBCL.

Materials and Methods

Patients and Samples

Formalin-fixed, paraffin-embedded tissue diagnostic samples from 64 patients with FL (cohort 2022 or C'22) were collected from 16 Spanish hospitals between 2006 and 2018. Twenty-three of the 64 patients underwent HT to DLBCL, and 41 remained nontransformed (Supplementary Fig. S1A, Supplementary Tables S1 and S2). HT was diagnosed when new symptoms appeared, and a new biopsy was performed to confirm the transformation to DLBLC. Transformation into a DLBCL was defined as the histologic progression into a high-grade lymphoma with a diffuse pattern. Patients diagnosed with FL were selected for this study according to the following criteria: grade 1 to 3A, excluding grade 3B cases, and a minimum follow-up of 5 years for patients without HT. Mutational data from FL diagnostic samples from 19 of 64 patients (P2022_51 to P2022_74, Supplementary Tables S1 and S2) have been previously published.[21] Median follow-up was 7 years (8 years for patients still alive) (range, 3.9-10.0 years), minimum follow-up for alive ntFL patients was 4.8 years. The mean time to HT for the whole series was 5.2 years (range, 0.16-10.0 years) and 2.9 years for transformed cases (0.16-5.8 years). Further details are provided in Supplementary Table S1.

Additionally, results from the previously published cohort (2018 cohort or C'18)[15] were used in this study. The C'18 cohort includes 19 cases diagnosed with FL who underwent HT to DLBCL and 21 cases without transformation (Table 1 and Supplementary Table S1). Patients included in this study were selected following the same criteria as the C'22 cohort. The median follow-up of the C′18 series was 6.7 years (8.0 years for live patients).

The study was approved by the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda (PI-67/14). It was conducted in compliance with the principles of the Declaration of Helsinki. All participants gave their signed informed consent for inclusion. Samples were collected, and clinical data were managed following standardized protocols to guarantee the quality of the samples and the confidentiality of donor data. After approval by the corresponding ethics committees, material and data from other centers were anonymously

Table 1
Summary of clinical features of follicular lymphoma whole cohort

Variables	C′18 and C′22						*P*
	Total		ntFL		pre-tFL		
	N	%	N	%	N	%	
Patients	104		62		42		
Female	64	61.5	35	55.0	29	69.0	.195
Age >60 y	45	43.3	28	45.2	17	40.5	.636
FLIPI	104		62		42		.001
High grade	29	27.9	10	16.1	19	42.2	
Low/intermediate grade	75	72.1	52	83.9	23	54.8	
Exitus	31	29.8	7	11.3	24	57.1	.001
ECOG	93		52		41		.045
0	52	55.9	35	67.3	17	41.4	
1	36	38.7	15	28.8	21	51.2	
2	5	5.4	2	3.8	3	7.3	
Stage	103		61		42		.086
I	7	6.8	7	11.5	0	0.0	
II	16	15.5	8	13.1	8	19.0	
III	30	29.1	15	24.5	15	35.7	
IV	50	48.5	31	50.8	19	45.2	
Firstline treatment	103		61		42		.161
R-CHOP	44	42.7	26	42.6	18	42.8	
R-Bendamustine	22	21.4	14	22.9	8	19.0	
Other IC (with R)	22	21.4	10	16.4	12	28.6	
Chemo	10	9.7	6	9.8	4	9.5	
W&W/radio	5	4.9	5	8.2	0	0.0	
Grade	83		50		33		.075
I	21	25.3	17	34.0	4	12.1	
II	40	48.2	22	44.0	18	54.5	
IIIA	22	26.5	11	22.0	11	33.3	
BCL2 rearrangement	57		40		17		.222
t(14;18) positive	40	70.2	30	75.0	10	58.8	
t(14;18) negative	17	29.8	10	25.0	7	41.2	

C′18, cohort 2018[15]; ECOG, Eastern Cooperative Oncology Group performance status; C′22, cohort 2022; FL, follicular lymphoma; FLIPI, Follicular Lymphoma International Prognostic Index; IC, immunochemotherapy; ntFL, nontransformed FL; pre-tFL, pre-transformed FL; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

transferred to our laboratory in compliance with the current Spanish legislation (Ley 14/2007 de Investigación Biomédica and Real Decreto 1716/2011).

DNA Extraction and Targeted DNA Sequencing

All samples were reviewed upon arrival to confirm the diagnosis and select tumor cell–enriched areas in the cases that required it. Further details are in the Supplementary Methods in the Supplementary Information.

We used 2 targeted sequencing panels (human reference genome GRCh38/hg38), including coding regions, splice sites and untranslated regions of genes recurrently altered in germinal center-derived B-cell lymphomas (Supplementary Table S3)[15,21,22]: SureSelect XT HS, Agilent Technologies, and Twist Bioscience. We analyzed the 44 genes that were common to the 2 panels. The probes designed for the common genes covered the same regions with the 2 systems (both based on hybridization and capture). They delivered similar results in terms of coverage and depth. Fifty DNA libraries were prepared with the SureSelect XT HS kit and 14 with the Twist Target Enrichment kit, as previously described.[21] Genomic data have been deposited in the Sequence Read Archive (BioProject ID PRJNA904556).

Bioinformatics Pipeline

A bioinformatic analysis was performed, and the genetic alterations were identified as previously described.[15,21] See Supplementary Information for further details.

Statistical Analyses

Time to transformation was defined as the duration of the period from the date of FL diagnosis to that of HT or last follow-up.

We used the Cox proportional hazards (PH) model for the univariate and multivariable analyses.[23] We did not consider the competing risks approach due to the small number of deaths in the nontransformed group; the external validation process in the competing risk setting is more complex to apply with the most used statistical approaches.

We developed the following 3 models: (1) we selected those genes with more than 4 mutations and performed a Cox univariate regression for each gene. Genes with a *P* value of <.10 were selected for inclusion in a multivariable Cox regression model derived by backward elimination and genes with a *P* value of <.05 were retained in the final "genetic" model; (2) in parallel, we developed the "clinical" model, which included FLIPI, by adopting the Cox PH method; and (3) we merged the genetic and clinical variables to obtain the "clinicogenetic" Cox PH model. We noted the corresponding Harrell's C-index, the Akaike information criterion (AIC), and the Bayesian information criterion (BIC) for each of the 3 models. AIC is a stastistical model based on in-sample fit to estimate the likelihood of a model to predict/estimate future values. BIC is another criterion for model selection that measures the trade-off between model fit and complexity of the model. A lower AIC or BIC value indicates a better fit.[24]

We compared the 3 features in the "clinical" and "clinicogenetic" models and selected the better model of the 2. The PH assumption was assessed by inspecting the Schoenfeld residuals. We also estimated the Brier score for the derivation and validation cohorts as an overall measure of fit.[25] The Brier score measures the accuracy of probabilistic predictions and may take a value between 0 and 1, with a smaller value indicating a better outcome.

The probability of HT at 2 or 5 years for an individual patient was calculated using the following equation, derived from the Cox PH model:

$$\widehat{P}\,(HT) = 1 - S_0(t)^{\exp\,(\text{Prognostic Index})}$$

where $S_0(t)$ is the baseline probability HT function at time t (ie, at 2 years), and the Prognostic Index is the sum of the products of the predictors and their coefficients.

For ease of interpretation, we developed a nomogram[26,27] that shows the risk of transformation at 2 and 5 years according to the variables retained in the final model. The internal validity of the model was assessed using a bootstrapping approach with 500 resamples. Two risk groups (low and high risk) were established based on the median value of the linear predictor[28] and illustrated graphically with Kaplan-Meier curves. The log-rank test was used to compare the 2 groups.

Calibration was evaluated through 2 calibration plots at 2 and 5 years. We also developed a Kaplan-Meier graph with each risk group's observed vs expected curves. Discrimination was assessed with Harrell's C-index.[29] The AIC and BIC were also calculated.

External validation was performed in the British Columbia Cancer (BCC) independent cohort,[14] which included mutational data from 246 FL diagnostic samples. We used the mutational and

Figure 1.
Genetic alterations identified in the tumor DNA samples of the C'22 cohort. The bar diagram represents the most frequently altered genes in patients who did (red) or did not (green) undergo histologic transformation.

clinical data from 146 cases. We excluded data from patients whose FLIPI or follow-up time information was unavailable (Supplementary Fig. S1B). The original publication did not provide individual follow-up times for ntFL patients. Still, according to their results, all these patients had a minimum OS (follow-up) of 7 years. The cohort included patients diagnosed with FL grade 1 to 3A, excluding grade 3B cases. Calibration was evaluated through a Kaplan-Meier curve showing the observed vs the expected curves for each risk group in the validation cohort. We also created a calibration plot for every follow-up year. Discrimination was assessed with Harrell's C-index.

Pearson's χ^2 test was used to compare categoric data between 2 groups.

Statistical analyses were performed using STATA v18 (StataCorp, 2023, Stata Statistical Software: Release 18: StataCorp LLC.).

Results

The C'22 cohort comprised 64 patients diagnosed with FL (see Materials and Methods section). There were 25 males (39.1%) and 39 females; 32 patients (50%) were older than 60 years at the time of diagnosis, and 15 (23.4%) had a high-risk FLIPI. Twenty-three of the 64 patients underwent HT to DLBCL, and 41 remained non-transformed (Table 1, Supplementary Fig. S1A, Supplementary Tables S1 and S2).

We performed a targeted deep-sequencing analysis in the C'22 cohort diagnostic FL samples as previously described.[15,21,22] A total of 520 alterations were identified, considering nonsynonymous mutations in the exonic regions and splice sites. The most frequently mutated genes were *KMT2D* (67.2%), *CREBBP* (65.6%), *BCL2* (46.9%), *TNFRSF14* (32.8%), *IGLL5* (26.6%), and *EZH2* (21.9%) (Fig. 1, Supplementary Table S4), with frequencies similar to those previously described in other series.[18,30,31] Some genes were more frequently mutated in pre-tFL samples, such as *EP300* (in 26% of the pre-tFL cases vs 12% of ntFL cases), *HIST1H1E* (17% vs 5%), *TP53* (13% vs 5%), and *CSMD3* (13% vs 2.5%). Other genes were more frequently mutated in ntFL samples, such as *TNFRS14* (in 17.4 % of the pre-tFL cases vs 41.5% of ntFL cases), *CREBBP* (56% vs 71%), *KMT2D* (61% vs 71%), and *EZH2* (13% vs 27%) (Fig. 1).

The study's initial aim was to validate the HT-predictive model previously generated by González-Rincón et al.[15] However, when we applied it to the C'22 cohort, no significant differences were found in the HT probabilities between the 3 risk groups (log-rank test, $P = .21$, based on the Kaplan-Meier analysis), probably due to the underpowered analysis performed before (Supplementary Fig. S2).

Therefore, we combined the C'22 cohort with the previously published C'18 cohort[15] to generate a new HT risk model. Thus, the new cohort included 104 cases, of which 62 were ntFL and 42 were pre-tFL (Supplementary Fig. S1A). The patient characteristics of the 2 cohorts are summarized in Table 1 and Supplementary Table S1.

We performed a Cox univariate regression to examine the genes mutated in at least 5% (5 cases) in the whole combined cohort (*CREBBP*, *TP53*, *PIM1*, *BCL2*, *CSMD3*, *CARD11*, *EP300*, *POU2AF1*, *NOTCH2*, *UBE2A*, *DTX1*, *KMT2D*, *ATP6V1B2*, *KMT2C*, *TNFRSF14*, *IRF8*, *IGLL5*, *HIST1H1C*, *DSP*, *SOCS1*, *MEF2B*, *PIK3CD*, *GNA13*, *ARID1A*, *STAT6*, *EZH2*, *B2M*, *EBF1*, *HIST1H1E*, *BCL7A*, *FOXO1*, *POU2F2*, *PAX5*, *ATM*, and *TNFAIP3*). The univariate analysis found that nonsynonymous mutations in the *CSMD3*, *NOTCH2*, *UBE2A*, *B2M*, and *HIST1H1E* genes were associated with a higher risk of HT, and mutations in *TNFRSF14* and *KMT2D* were associated with a lower risk of HT ($P <$ .10) (Fig. 2A). A multivariate Cox regression model initially including all these genes was derived by backward elimination. The results showed that mutations in *HIST1H1E* and *B2M* were significantly associated with a higher risk of HT, and mutations in *TNFRSF14* and *KMT2D* were linked to a lower risk of HT ($P < .05$) (Fig. 2B). These 4 genes comprise the final genetic model.

We used univariate PH Cox analysis to estimate the associations of clinical characteristics with HT. High-risk FLIPI[32] (FLIPI score ≥ 3) was found to be associated with HT (HR, 3.046; 95% CI, 1.652-5.615; $P < .001$), but no significant association was found with gender, stage, age >60 years, or histologic grade. ECOG was not analyzed, because there were so few patients with an ECOG >1 ($n = 5$). Therefore, the FLIPI was the only variable in the clinical model.

For the final clinicogenetic histologic transformation risk (HTR) model, we combined the FLIPI and genes in the genetic model and finally included FLIPI and *HIST1H1E*, *KMT2D*, and *TNFSR14* genes (Supplementary Table S5). The clinicogenetic HTR model was slightly, but not significantly, better than the genetic model (C-indices: 0.746 vs 0.704; $P = .297$) but significantly better than the FLIPI (C-indices: 0.746 vs 0.618; $P < .001$). The AIC and BIC values also indicated that the clinicogenetic HTR model was better than the others (Table 2). Brier scores were also calculated. At 2 years, these were 0.098 for the clinicogenetic HTR model and 0.121 for the FLIPI. The 5-year corresponding values were 0.175 and 0.217, respectively (Table 2).

A nomogram was then constructed based on the HTR model to predict the risk of transformation at 2 and 5 years (Fig. 3A). The calibration plots showed good agreement between the 2- and

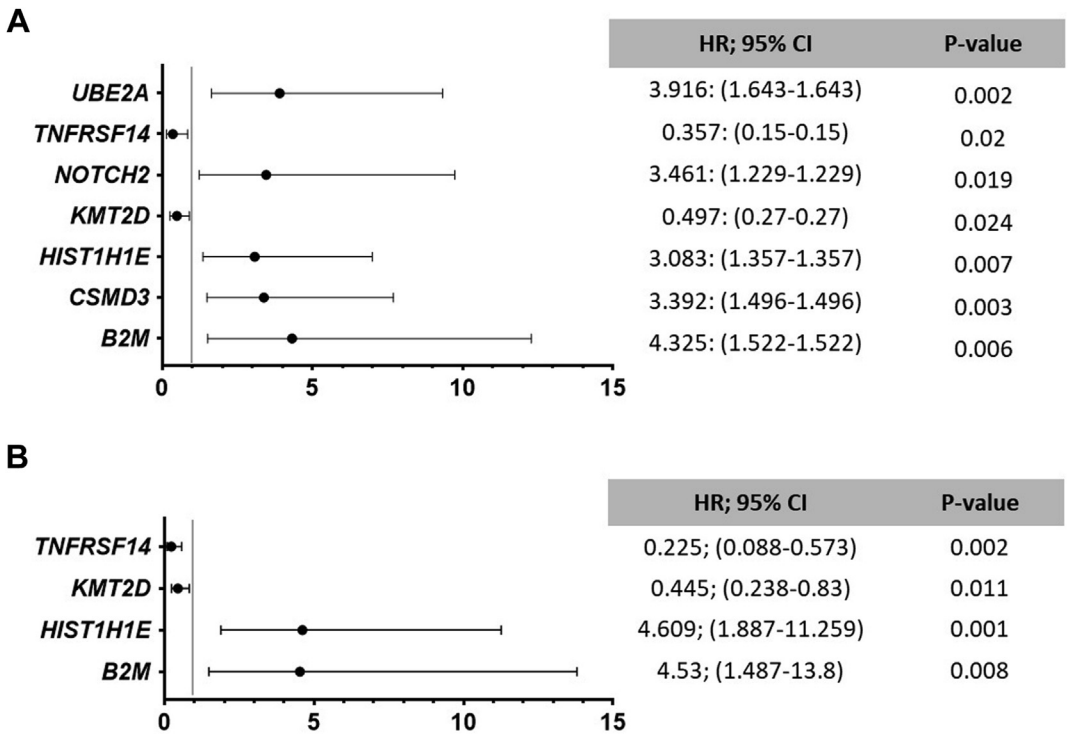

Figure 2.
Forest plots of (A) univariate and (B) multivariate Cox regression analysis of the effect of mutated genes of histologic transformation in follicular lymphoma patients.

5-year predicted and observed risk of HT (Fig. 3B, C, Supplementary Fig. S4A).

The probabilities of HT at 2 and 5 years for an individual patient were calculated using the following equations, derived from the Cox PH model:

$$\widehat{P}\ (\text{HT at 2 years}) = 1 - 0.9954^{\exp\ (\text{Prognostic Index})}$$

$$\widehat{P}\ (\text{HT at 5 years}) = 1 - 0.9858^{\exp\ (\text{Prognostic Index})}$$

where Prognostic Index = 1.639193 * FLIPI (high-risk) + 1.383305 * *KMT2D* (not mutated) + 1.615207 * *TNFRSF14* (not mutated) + 2.102908 * *HIST1H1E* (mutated). A Web-based calculator is available at https://github.com/Lymphoma-IDIPHISA.

We internally validated the HTR model by bootstrapping with 500 resamples. Discrimination was evaluated using Harrell's C and Somers' D indices,[29] which indicated that the model had a good discriminating ability (Harrell's C = 0.746; Somers' D = 0.492).

Patients were classified into low- and high-risk HT groups based on the median value of the linear predictor.[28] The Kaplan-Meier time to HT analysis revealed that patients in the HT high-risk group had a significantly shorter time to transformation than those in the low-risk group (Fig. 4A, Supplementary Fig. S5A). The HT time-to-event probability at 24 months in the low-risk group was 0.90 (95% CI, 0.81-0.95) vs 0.51 (95% CI, 0.24-0.73) in the high-risk group; and at 60 months it was 0.71 (95% CI, 0.61-0.80) and 0.15 (95% CI, 0.02-0.37), respectively.

Finally, we validated the model in an external cohort. We used the BCC cohort[8] described in Materials and Methods (Supplementary Fig. S1B, Supplementary Table S7). We applied the HTR model to 146 cases (65 pre-tFL and 81 ntFL) after discarding cases for which the FLIPI or follow-up time information was unavailable. The calibration plots in the validation cohort also showed good agreement between the predicted and observed 1- to 7-year risk of HT (Supplementary Fig. S3B). When we applied the nomogram and divided the series into low- and high-risk groups as described before, the Kaplan-Meier analysis revealed a significantly shorter time to transformation among the high-risk group of patients than the low-risk group (Fig. 4B, Supplementary Fig. S4B). The HT time-to-event function at 24 months in the low-risk group was 0.86 (95% CI, 0.79-0.91) vs 0.54 (95% CI, 0.32-0.72) in the high-risk group. At 60 months, the values were 0.71 (95% CI, 0.62-0.78) and 0.32 (95% CI, 0.14-0.51), respectively.

The values of Harrell's C-index (0.552) and Somers' D (0.105) pointed to a fair level of discrimination in the external validation cohort. The Brier score for the validation cohort was 0.138 (95% CI, 0.099-0.178) at 2 years and 0.208 (95% CI, 0.178-0.238) at 5 years.

Table 2
Harrell's C-index, the Akaike information criterion, the Bayesian information criterion, and the Brier scores for the 3 histologic transformation risk models

Models	C-index	AIC	BIC	Brier score 2 y (95% CI)	Brier score 5 y (95% CI)
Genetic	0.704	345.834	356.411		
Clinic (FLIPI)	0.618	355.836	358.481	0.121 (0.076-0.166)	0.217 (0.179-0.256)
Clinicogenetic	0.746	331.635	342.213	0.098 (0.056-0.140)	0.175 (0.132-0.218)

AIC, Akaike information criterion; BIC Bayesian information criterion; C-index, Harrell's C-index; FLIPI, Follicular Lymphoma International Prognostic Index.

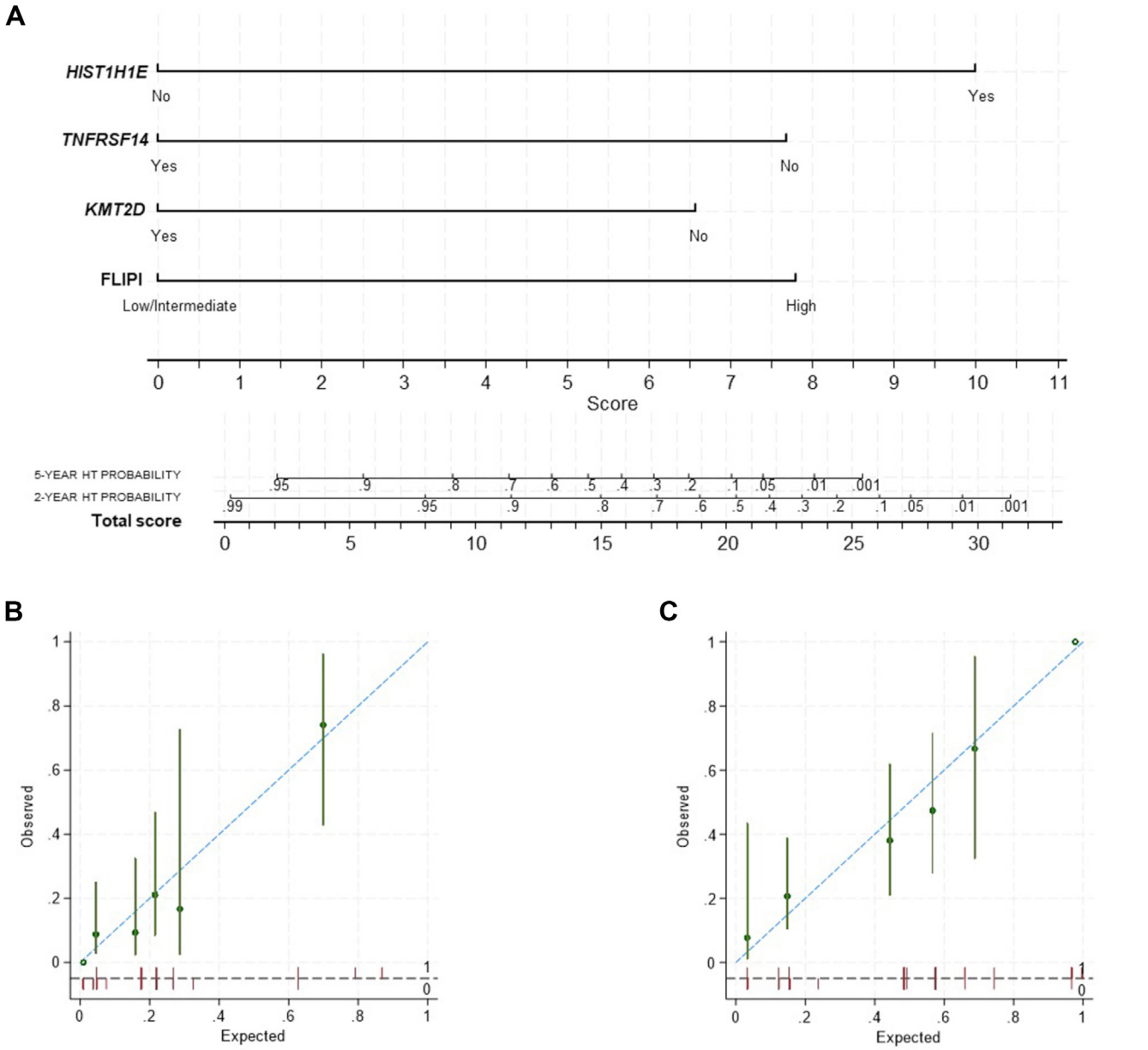

Figure 3.
(A) Nomogram estimating 2-year and 5-year probability of follicular histologic transformation to diffuse large B-cell lymphoma. (B) Calibration curve of the nomogram estimating histologic transformation probability for follicular lymphoma patients at 2 years and (C) 5 years. Error bars = 95% CIs. Blue dashed diagonal lines represent the perfect calibration models with identical predicted and observed probabilities.

Discussion

Transformation to an aggressive lymphoma is a critical event in the clinical course of FL patients. It has been associated with a worse prognosis than that of patients who do not experience HT.[6] Additionally, transformation <2 years after diagnosis is associated with shorter survival than that in patients who undergo later transformations.[33] Therefore, predicting HT at the time of diagnosis is a valuable clinical task. Several clinicopathologic factors have been associated with a higher risk of HT, such as a high FLIPI, advanced stage, ECOG $\geq$ 2, and grade 3A.[7,34] Some individual genetic alterations have also been associated with HT, such as *TP53* mutations, *MYC* alterations, *CDKN2A* loss by deletion or promoter hypermethylation, and other factors.[14,15,17,18] Several attempts have been made to predict clinical outcomes of FL patients by combining clinical factors and mutations in multiple genes, such as m7-FLIPI, to predict failure-free survival,[35] and POD24-PI, to predict early progression after firstline immunochemotherapy.[36] However, their predictive value differs between series, and none of them can predict HT.[6,35,37] Our previous analysis led us to propose a mutational signature associated with a shorter time to transformation.[15] However, we still lack an accurate tool that can predict transformation at diagnosis.

In this study, we propose a new clinicogenetic model to predict FL risk of HT to DLBCL based on the FLIPI and the mutational status of 3 genes. We used a nomogram to develop an applicable method

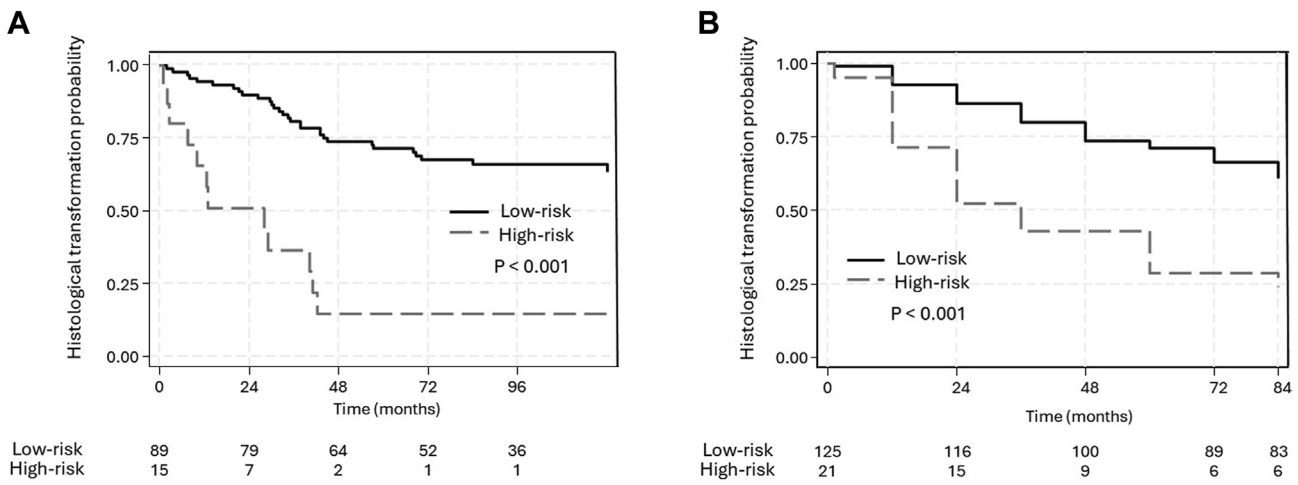

Figure 4.
Kaplan–Meier histologic transformation time-to-event estimates. (A) Time to transformation for the combined C′22 and C′18 cohort, and (B) for the British Columbia Cancer validation cohort, stratified into low-risk and high-risk groups by the median value of the linear predictor.

that could predict individual risk of HT to DLBLC at 2 and 5 years, taking into consideration the FLIPI and the mutational data of *HIST1H1E*, *KMT2D*, and *TNFSR14*.

HIST1H1E is a chromatin-remodeling gene whose alterations have been identified as clonal in the FL samples and have been previously associated with HT.[14,15,17] A recent study by Yusufova et al[38] analyzed the effect of H1 lymphoma-associated alterations in a mouse model that includes *HIST1H1E*. They showed that loss of *HIST1H1E* and *HIST1H1C* bestowed enhanced self-renewal capacity on germinal center B cells, which prompted the development of aggressive lymphomas.

KMT2D (also known as *MLL2*) encodes an H3K4 trimethyltransferase. Loss-of-function mutations, which frequently occur in this gene, are clonal, ancestral, driver mutations in FL that give rise to B-cell lymphomagenesis.[39,40] *KMT2D* is the most commonly mutated gene in our cohort, having been noted as occurring in up to 80% of cases in 2 earlier studies.[18,30] This figure is higher than that recorded in DLBCL.[22,41,42] The relationship of *KMT2D* with B lymphomagenesis indicates a possible dependence on the loss of function of *KMT2D* in FL tumors but less so in DLBCL, supporting the hypothesis that *KMT2D* gene alteration limits the ability of FL to acquire additional genetic alterations for HT. However, although we found an inverse association with HT, the mechanism responsible has not been investigated.

However, the role of *TNFRSF14* alterations is controversial in lymphomas. *TNFRS14* encodes herpes virus entry mediator, which is known to activate the NF-κB pathway, leading to the induction of proinflammatory and cell survival–promoting genes, but which can function as a "molecular switch" with activating or inhibitory functions.[43] Loss of *TNFRSF14* in mouse models leads to cell-autonomous activation of B-cell proliferation, which drives the development of GC lymphomas.[44] Some studies found an association of *TNFRSF14* mutations with shorter OS and worse prognosis,[45,46] whereas others found *TNFRSF14* mutations and deletions (1p36.23-1p36.32) to be associated with better prognosis[47,48] or to be more frequently mutated in ntFL than in pre-tFL cases.[15] Mutations in *TNFRSF14* are common in a subgroup of t(14;18)-negative FLs with inguinal localization, which are linked to a localized presentation and a more indolent disease.[49] But *TNFRSF14* is also frequently mutated in classic t(14;18)-positive FL cases.[46,50] We have found *TNFRSF14* mutations in 32 of 104 cases. Our series included (14;18) positive and t(14;18) negative FL (Table 1), and *TNFSRF14* mutations were detected in both subgroups (12 and 4 FL, respectively).

A recent study by Dreval et al[19] has shown that mutations in *TNFRS14* and *KMT2D* genes, among others, were more frequently detected in non-HT cases, which is consistent with our study.

This new clinicogenetic model could pave the way to HT prediction by combining genetic alterations with clinical features. HT stratification could be improved by identifying 2 risk groups, leading to a higher proportion of HT cases being assigned to the high-risk and lower-risk groups, more accurately than solely with FLIPI.

We have internally cross-validated the model and validated it in an external cohort. Nevertheless, our study has limitations because it is based on retrospective data of patients, some of whom were diagnosed as early as 2006. We recognize that more recent series might have behaved differently. Therefore, further clinical validation of the model in prospective series is needed to be eventually used as an HT risk predictor. Additionally, the immune microenvironment of FL is being extensively investigated, and the different tumor microenvironment immune compositions in different samples may influence the path of tumor evolution and the clinical course. Integrating genetic alterations and microenvironment composition should give us a better understanding of the HT process and allow us to identify integrative signatures that can predict HT risk and could eventually be used as biomarkers for clinical decisions regarding treatment and follow-up.

In conclusion, we have built and externally validated a clinicogenetic HT risk model, combining mutation information from only 3 genes (*HIST1H1E*, *KMT2D*, and *TNFRSF14*) with clinical features at diagnosis (FLIPI). Predicting the risk of HT is relevant for patients with FL, improving their clinical management and allowing treatment strategies that could prevent or delay transformation.

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Author Contributions

M.S.-B., I.F.-M., and M.P. contributed to conception and design. M.S.-B. and M.P. made financial support. M.S.-B. was involved in administrative support. B.E., F.dl.C., F.C., F.I.C., P.M.-A., M.M.L.C., A.S., M.G.-C., M.P.-V., M.L., J.F.G., S.S., S.M., S.G.-H., B.N., M.M., and M.P. were involved in provision of study materials and patients. I.F.-M., L.P., J.G.-R., S.G., N.Y.-C., M.D., B.H., and M.N. contributed to collection and assembly of data. I.F.-M., A.R., M.P., and M.S.-B. were involved in data analysis and interpretation. I.F.-M. and M.S.-B. contributed to manuscript writing. All the authors critically reviewed and approved the final manuscript, and are accountable for all aspects of the work.

Data Availability

Genomic data have been deposited in the Sequence Read Archive (BioProject ID PRJNA904556).

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Declaration of Competing Interest

None declared.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda (PI-67/14). It was conducted in compliance with the principles of the Declaration of Helsinki. All participants gave their signed informed consent for inclusion. Samples were collected, and clinical data were managed following standardized protocols to guarantee the quality of the samples and the confidentiality of donor data. After approval by the corresponding ethics committees, material and data from other centers were anonymously transferred to our laboratory in compliance with the current Spanish legislation (Ley 14/2007 de Investigación Biomédica, Real Decreto 1716/2011 and Ley Orgánica 3/2018).

Supplementary Material

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