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DEVELOPMENT OF A COMPREHENSIVE PROGNOSTIC INDEX FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

Prognostication in CLL

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KEYPOINTS

- A prognostic tool for CLL patients with high discriminatory power in comparison to the conventional clinical staging systems
- Allowing prognostication on the individual patient level independent of clinical stage

ABSTRACT

Besides clinical staging, a number of biomarkers predicting overall survival (OS) have been identified in chronic lymphocytic leukemia (CLL). The multiplicity of markers, limited information on their independent prognostic value, and a lack of understanding of how to interpret discordant markers are major barriers to use in routine clinical practice. We therefore performed an analysis of 23 prognostic markers based on prospectively collected data from 1948 CLL patients participating in phase III trials of the German CLL Study Group to develop a comprehensive prognostic index. A multivariable Cox regression model identified eight independent predictors of OS: sex, age, ECOG status, del(17p), del(11q), *IGHV* mutation status, serum β_2-microglobulin, and serum thymidine kinase. Using a weighted grading system, a prognostic index was derived that separated four risk categories with 5-year OS ranging from 18.7% to 95.2% and having a *C*-statistic of 0.75. The index stratified OS within all analyzed subgroups, including all Rai/Binet stages. The validity of the index was externally confirmed in a series of 676 newly diagnosed CLL patients from Mayo Clinic.

Using this multi-step process including external validation, we developed a comprehensive prognostic index with high discriminatory power and prognostic significance on the individual patient level.

Studies were registered as follows: CLL1 trial (NCT00262782), http://clinicaltrials.gov; CLL4 trial (ISRCTN 75653261), http://www.controlled-trials.com; and CLL8 trial (NCT00281918), http://clinicaltrials.gov.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia of the western world, with an incidence of 4.1/100,000 per year.[1] The disease displays a high heterogeneity in its clinical course.[2,3] Patients presenting with an indolent form often do not require treatment for decades, while others experience a very aggressive course leading to death within months.

Currently, staging and prognostication of CLL is performed by two similar clinical staging systems developed 30-35 years ago by Binet et al.[4] and Rai et al.[5]. Both systems use inexpensive, simple components such as blood counts and physical examination to identify three major prognostic subgroups. Despite these advantages, the clinical staging systems do not fully reflect the high variability of CLL, nor do they account for known biologic characteristics of CLL cells predicting survival and response to therapy.[3,6,7]

Recently an impressive array of novel effective therapies has been developed which holds the potential of increasingly individualized treatments if patient risk could be accurately characterized.[8-10] Unfortunately, the large number of novel prognostic markers in CLL, limited information on their independent prognostic value, and a lack of understanding of how to interpret discordant markers are still major barriers to integrate these in routine clinical CLL practice.[11] To address this issue, we used the German CLL Study Group database to conduct a comprehensive evaluation of 23 clinical, biological and genetic markers in CLL. The aim was to develop a prognostic index that identifies and combines the

prognostic markers of independent importance that are already available. The utility of the developed prognostic index was subsequently validated using an external cohort of newly diagnosed CLL patients from the Mayo Clinic, Rochester, MN, USA.

METHODS

Study population

Data from three prospective randomized phase III trials conducted between 1997 and 2006 by the GCLLSG were used as a training dataset. All patients were untreated and had a diagnosis of CLL according to NCI Working Group Criteria[12].

The CLL1 trial[13] (NCT00262782) included 876 Binet stage A patients and compared a watch-and-wait (W&W) strategy to early fludarabine (F) monotherapy in patients having high risk for progression. The CLL4 trial[14] (ISRCTN 75653261) included 375 patients younger than 65 years requiring treatment and compared F to F plus cyclophosphamide (FC). The CLL8 trial[15] (NCT00281918) included 817 patients in need of treatment comparing FC to FC plus rituximab (FCR). Patients who were initially allocated to W&W in CLL1 and received first-line treatment later within CLL4 or CLL8 (N=61) were only accounted for once. For those, data from first presentation (CLL1) was considered including the longest observation period and corresponding baseline values.

All trials were approved by the leading ethics committee. Written informed consent was obtained according to the Declaration of Helsinki.

Data Collection

Pretherapeutic features evaluated for potential prognostic relevance were sex, age, time between diagnosis and registration/randomization, Binet/Rai stages, ECOG performance status (PS)[16], B-symptoms, blood counts, genetic abnormalities[17,18], expressions of ZAP-70/CD38[19,20] , *IGHV* mutation status (MS)[21 20,22,23], serum lactate dehydrogenase (LDH), serum thymidine kinase (s-TK)[24-27], and serum β_2-microglobulin (s-β_2m)[24,28,29]. S-TK and s-β_2m were evaluated centrally; s-β_2m was analyzed by immunometric chemiluminescence assay and s-TK by either radioimmunoassay or quantitative immunoassay, respectively. Leukemic cells isolated from the peripheral blood were used for the determination of *IGHV* MS and assessment of ZAP-70/CD38 expression. Detailed descriptions of the diagnostic methods have been published previously.[17,19,30,31,32] Data on ZAP-70/CD38 were not available for CLL1.

Statistical Analysis

The main endpoint of statistical analyses was OS defined as the time between registration/randomization and death. Treatment free survival (TFS) and progression free survival (PFS) were calculated from registration/randomization to start of first CLL treatment or from registration/randomization to disease progression or death, respectively. Subjects without a documented event were censored at time of last follow-up. Survival rates and standard errors were estimated by Kaplan-Meier methods[33], survival curves were compared using log-

rank tests. The prognostic relevance of each factor was evaluated applying Kaplan-Meier methodology and Cox proportional-hazards regression analyses.[34] Continuous biologic variables were dichotomized using published thresholds, laboratory norms and quartiles. Threshold analysis including ROC curves[35] and Youden Index[36] were applied to identify additional thresholds. Dichotomized variables were only considered for further analysis if the continuous analogue was of prognostic importance in univariate proportional-hazards Cox regression.

All variables that showed significant association with OS on univariate analysis were consequently included in multivariate analysis applying forward and backward stepwise proportional-hazards Cox regressions.

The analysis was further controlled for the variables “study” (CLL1/CLL4/CLL8), “type of first-line treatment” (W&W/F/FC/FCR), treatment indication status, B-symptoms, time between diagnosis and registration/randomization, lymphadenopathy, spleno-/hepatomegaly, hemoglobin, lymphocyte and platelet counts to account for possible treatment effects as well as for the heterogeneous dataset consisting of patients with and without treatment indication. For testing interactions in the final model, the multivariate analysis was repeated including the independent factors, the variable “type of first-line treatment” as well as terms for interactions between factors and treatment.

Robustness of the multivariable Cox model was verified by bootstrapping techniques.[37-39] A complete case analysis was applied to avoid the problem of missing data.

Factors independently associated with OS in the final model were included in the prognostic index. To account for differences in the magnitude of association between the individual independent factors and OS, we assigned a weighted risk score to each factor based on ranges of their corresponding hazard ratios. The total risk score was then calculated by the sum of the ratings of individual factors. To identify risk groups, the following criteria for the combination of risk categories were defined: i) statistically significant differences in OS of risk groups, ii) absence of heterogeneities concerning independent factors within each risk group and iii) adherence of smallest loss of information in terms of log-likelihood change. *C*-statistics were calculated to further evaluate discriminatory value of the prognostic index (c=1 indicates perfect discrimination; c=0.5 equivalent to chance).[37,40] All tests were two-sided and significance was defined as a p-value of <0.05. The analyses were performed using SPSS Statistics 21.

External Validation

The validation dataset was comprised of a consecutive series of 676 newly diagnosed prospectively followed CLL patients cared for at Mayo Clinic who had baseline data on all considered variables except s-TK and/or s-β_2m available and who had stored serum collected ≤36 months (median: 1 month) of diagnosis available for s-TK and s-β_2m analysis. Stored serum was shipped to the Institute for Clinical Chemistry at the University Hospital of Cologne for subsequent s-TK assessment. Since the s-TK assays in the training dataset measured s-TK using

a radioimmunoassay and the validation cohort used a non-radio labeled immunoassay, inter-assay calibration of both assays was performed (correlation R^2=0.89) to allow mathematical conversion before applying the s-TK threshold for assigning index point score. For the validation cohort OS was defined as the time between diagnosis and death. TFS was calculated from date of diagnosis to start of first CLL treatment. Subjects without a documented event were censored at time of last follow-up. The outcome of individuals was prospectively assessed.

RESULTS

Patients' characteristics of the training dataset

After excluding patients with missing baseline data (N=47), and those with insufficient follow-up (N=12), 1948 eligible patients were available as a training dataset (flow chart, Supplemental Material I). Median age was 60.0 years (range, 30.0-81.0); 485 deaths from all causes were reported after a median observation time of 63.4 months.

Patient characteristics are shown in Table 1A. Survival curves according to Binet/Rai stages are shown in Figures 1A/B.

Univariate and multivariate analyses

Except for ZAP-70/CD38 status, and del(6q), all parameters showed a significant correlation with OS using univariate analysis (Table 1A) and variables were subsequently considered for multivariate analysis.

Eight parameters were identified as independent predictors of OS in 1223 patients with all parameters significant on univariate analysis available: sex, age, ECOG PS, genetic aberrations del(17p) and del(11q), *IGHV* MS , s-TK, and s-β_2m (Table 2). These 1223 patients were representative of the entire population training dataset. All variables used to control for possible confounding effects - as specified in the Methods section - were proven not to be independent factors for

OS. Internal validation was performed by bootstrapping techniques: Based on 100 generated resamples of the training dataset, regressions were repeated, and the robustness of the 8-parameter model was confirmed uniquely (Table 2).

We also repeated the multivariate analysis analyzing CLL1 (an early intervention trial) and CLL4/8 (first-line treatment trials) separately (see Supplement Material Figures 4 and 5). The key molecular biomarkers/serum factors identified for inclusion in the model (s-TK, s-β_2m, *IGHV* MS, del(17p)) were similar in both models. Notably, no other/unique molecular characteristics were identified for either models with the exception of deletion 11q23 which was significant in the CLL1 model but not in the CLL4/8 model (potentially due to small sample size and inadequate power). These findings provide support for pooling the data from the CLL1, CLL4, and CLL8 trials to determine whether additional factors enter the model with larger sample size and greater power.

Prognostic Index

Next, given a large range of hazard ratios (HR) of the independent factors (e.g. HR=1.3 for sex; HR=6.0 for del(17p)), a risk score was assigned to each of the independent factors in the final model (Table 2). The weighting was based on a simple algorithm assigning the integer value of the corresponding HR to each factor (i.e. 1 point for HR 1.1-1.9; 2 points for HR 2.0–2.9, etc.). Finally, we defined the total risk score as the sum of the risk scores of the 8 individual factors (range, 0-14).

According to the predefined criteria (see Methods section) four different risk categories for OS were determined: low (score 0-2, N=300), intermediate (score 3-5, N=460), high (score 6-10, N=410), and very high (score 11-14, N=53) (Table 4). The proposed risk categories segregated 5-year OS rates from 95.2% (low risk) to 18.7% (very high risk) ($p<0.001$) with c=0.75 (Table 3 and Figure 2A).

Analyses for PFS in treated patients (N=807) and TFS in patients managed with W&W (N=416) also demonstrated validity of the prognostic index for these endpoints. Among treated patients, 5-year PFS rates of the four risk groups were 62.9%, 43.6%, 25.6% and 6.4%, respectively ($p<0.001$; Figure 2B). Among patients initially managed with W&W, 5-year TFS rates were 86.2%, 52.4%, 22.1% and 0.0% respectively ($p<0.001$; Figure 2C).

Subgroup analyses corroborated the discriminative strength of the prognostic index. The four risk groups were reproduced within each Binet/Rai stage ($p<0.001$, respectively) (Figure 3) and within *IGHV* unmutated patients (Figure 4A). Within the group of patients with del(17p), the index was able to distinguish patients with high risk from patients with very high risk ($p<0.001$) (Figure 4B).

External Validation

The utility of the prognostic index was subsequently evaluated in a prospectively followed validation cohort of 676 newly diagnosed CLL patients cared for at Mayo Clinic (Table 1B). Three patients were excluded due to missing data for s-β_2m. Median observation time was 57.0 months and 85 deaths (12.6%) were

observed. Median age was 61.5 years (range, 32.0-89.0). Within this cohort the 5-year OS for the respective risk groups were 95.2%, 91.4%, 71.7%, and 13.6% ($p<0.001$, Table 4, Figure 5A) (*C*-statistic: c=0.83).

At last follow-up 486 patients (71.9%) were still untreated and the validity of the prognostic index for predicting TFS was also confirmed: after 5 years, 77.1%, 55.7%, 23.9% and 0.0% respectively were untreated ($p<0.001$; Figure 5B).

The discriminative strength of the prognostic index within clinical stages (Figure 6) and biologic risk groups (Figure 7) was confirmed in the validation dataset, too.

Comparisons to the Prognostic Index

Wierda and colleagues[29] have proposed a prognostic model for OS including 3 factors contained in our model (age, s-β_2m, sex) but without genomic aberrations, *IGHV* MS, and s-TK. To explore how our index improved on this model, we identified 1144 patients in our training dataset with the necessary variables to classify patients according to both systems. The *C*-statistic of the previous model was c=0.61, a level below that of the full index (c=0.75) and below the accepted 0.7 threshold necessary to have value at the individual patient level.[40] When the incremental prognostic value of genomic aberrations, *IGHV* MS, and s-TK was assessed, each of these parameters improved prediction of OS compared to the previous model[29] without these factors:

del(17p) (HR=5.6 (95% confidence interval (CI), 4.0-7.8), p<0.001), del(11q) (HR=1.5 (95% CI, 1.1-2.0), p=0.005), *IGHV* MS (HR=2.1 (95% CI, 1.6-2.7); p<0.001), s-TK>10.0 U/L (HR=2.5 (95% CI, 1.8-3.4); p<0.001). In addition, classifying the patients of each risk strata (low, intermediate, high) of the previous model[29] according to our prognostic index provided substantial improvement in prognostication (Supplemental Material II).

DISCUSSION

For almost 40 years, the Rai/Binet staging classifications have formed the backbone of CLL management. However, it has become apparent that both systems lack precision in discriminating prognostic subgroups of CLL patients and that the ability to predict outcomes for individual patients is limited, as demonstrated by a *C*-statistic of only c=0.56 and c=0.58 for Rai- and Binet-staging respectively within our training dataset.[11,41] Furthermore, a multitude of new prognostic markers have been identified in the past decades. The aim of this analysis was to identify markers that have independent prognostic value among assays in routine clinical practice in the US and/or Europe. We also sought to determine how these factors can be combined into an integrated prognostic model that allows clinicians to interpret and apply the collective results of prognostic tests for individual patient counseling and to enable clinical scientists to develop risk-adapted therapies for clinical testing. The manuscript represents a major step forward of integrating the most important prognostic tools of the last 30 years into a single model. The lack of such an accurate prognostic system is

currently a major clinical problem in CLL.

We demonstrated in the training dataset and further confirmed in the validation dataset that the prognostic index developed allows a substantial gain of information compared to the conventional clinical staging systems as well as to the most important single risk factors known (unmutated *IGHV* MS and 17p deletion). The refined prognostic information provided by the index may have potential future application in identifying patients whose projected survival merits alternative or more aggressive treatment approaches (e.g. allogeneic stem cell transplantation), identifying early stage patients who are candidates for trials evaluating the benefits of early intervention/treatment and/or establishing risk-stratified treatment approaches with new emerging therapies. Relevant clinical phase III trials using this prognostic score for risk stratification of clinically early stage CLL patients are currently being conducted. These and other consecutive trials based on the prognostic index will probably lead to a refined and individualized treatment-algorithm in CLL.

We used a well-characterized and prospectively followed population of untreated CLL patients as a training dataset to construct a weighted, multivariable prognostic index which includes clinical, biological and molecular markers and defines four different risk groups with significantly different OS. These four risk groups were reproduced within each Rai/Binet stage and within the subset of patients with unmutated *IGHV* status or with del(17p) demonstrating the gain of information over the conventional clinical staging systems. The *C*-statistic of the model was c=0.75 exceeding the threshold level of 0.70 signifying prognostic

utility at the individual patient level.[40]

The utility of the prognostic index was also confirmed in an independent validation cohort. Although slight differences in projected survival rates were observed between training and validation datasets, these differences are likely due to a shorter observation time and high proportion of censored data (87.4%) in the validation cohort. However the *C*-statistic of the model in the validation cohort was c=0.83 and analyses of PFS and TFS - which can be seen as disease-specific outcomes and surrogate markers for OS - robustly confirmed the validity and potential of the prognostic index in both cohorts.

We further identified a “very high risk” group among CLL patients with an OS after 5 years of only 13.6-18.7%. This very high risk group comprises only 4% of CLL patients. Although all patients in this risk group are 17p deleted it should be emphasized that not all patients with 17p deletion are in this category. Specifically, patients with deletion 17p can be stratified into two risk groups (“high risk” or “very high risk”) with very different OS as illustrated in Figures 4B and 7B (p=0.001 and p=0.04). This finding once again demonstrates the discriminatory power of our index even in patients traditionally considered as high risk.

Recurring gene mutations affecting ~10-15% of CLL patients such as *NOTCH1* and *SF3B1* were recently identified by new generation sequencing.[42] Although controversial in the currently reported literature, these markers may have prognostic value.[43-48] Prospective clinical trials evaluating the significance of those markers for OS and the additional information in combination with clinical,

biological and genetic markers in CLL are further needed. Novel prognostic markers will continue to be discovered and accurate risk stratification needs to be an evolving process. The intent of the model presented here is to determine what existing clinical markers have independent value and to consolidate the prognostic value of these markers into a single risk score. Like the historical staging systems that combined clinical and laboratory data, such a platform facilitates evaluation of newly discovered markers - whether they offer incremental improvement over current knowledge. Of note prognostication with more traditional markers according to the here proposed classification seems to separate different risk groups more accurately than risk classification based on genetic characteristics exclusively.[46] While 6 of the 8 factors in the comprehensive prognostic model are widely available, *IGHV* MS and s-TK are not routine clinical assays at many centers. Therefore we evaluated whether we could eliminate these factors from the index, or whether a different model could be developed if *IGHV* MS and/or s-TK were not included in the initial 23 factors considered. In all cases the prognostic value of the index was reduced or lost altogether (data not shown) indicating that the risk measured by s-TK and *IGHV* MS are distinct from the other parameters. Nonetheless, *IGHV* analysis is already a routine assay at many clinical sites in both Europe and the USA. Similarly, s-TK is also widely available as routine clinical assay in some European countries and is in the process of being evaluated in American research laboratories as well.[26] It is therefore evident that clinical assays assessing these variables are both feasible and indeed already available. Therefore the

manuscript helps to eliminate unnecessary tests that do not provide incremental value. Now that the markers with the greatest independent prognostic value are identified, enhanced emphasis can be placed on making these markers more widely available in routine practice rather than developing clinical assays with lower relative value (e.g. ZAP-70/CD38[49-51]).

While attempts to create prognostic models for CLL have been made previously, none of these models incorporated this broad spectrum of markers or derived a risk score from a large prospectively followed patient cohort. Recently, a multivariable model for OS was developed[29], but without the most robust prognostic factors in CLL (e.g. genomic aberrations, *IGHV* MS). This model reached a *C*-statistic of c=0.61 only in our dataset below the value needed for clinical utility or relevance to an individual patient.[40] Other models using a full array of genetic characteristics (e.g. FISH testing in combination with sequencing of *TP53, NOTCH1, SF3B1,* and *BIRC3*) have generated *C*-statistics of c=0.642 only.[46] Our analysis suggests that harnessing the full array of clinical, serum and molecular characteristics optimizes the accuracy of OS prediction and that, for the first time, the comprehensive index presented here classifies risk accurately enough to be considered potentially useful for the individual patient (c=0.75-0.83).[40]

We are aware of some limitations of our analysis. Although our training and validation dataset included ~300 patients >age 70, the median age of 60 years in the training set is rather young compared to the reported median age at diagnosis of 72 years. Elderly patients are generally underrepresented in clinical

trials such as those from which the index was derived.[52] Although the clinical validation cohort from the Mayo Clinic was also somewhat younger than CLL patients on average, the prognostic index was found to reliably predict outcome of CLL patients from around the world, including newly diagnosed patient cohorts. Nonetheless, we recognize that further validation in an extended dataset including a larger sample of older, unfit patients is warranted. Further the fact that choice of therapy was not an independent prognostic factor for OS in the analysis should be interpreted cautiously since our analysis was not designed to extensively investigate the role of different treatment modalities.

In conclusion, we report the development and validation of a novel prognostic index for CLL patients which identifies clinical, serum, and molecular markers with independent prognostic value and combines them into a single risk score. To our knowledge, the index presented here is the first comprehensive prognostic model to simultaneously incorporate a broad spectrum of prognostic markers into a single prognostic index and to reach the C-statistic threshold ($c>0.70$) necessary to have utility at the individual patient level. The index appears broadly applicable, dramatically improves the accuracy of prognostication over classical CLL clinical staging systems and holds the potential for the development of more individualized treatment strategies. Clinical trials translating the information gained through application of the prognostic index into new refined treatment-algorithms for CLL patients are currently conducted.

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Previous Presentations

This work was presented in part at the 14th International Workshop on CLL (Houston, TX, USA, 2011), the 53rd Annual Meeting of the American Society of Hematology (San Diego, CA, USA, 2011) and the 119th congress of the German Society of Internal Medicine (Wiesbaden. Hessen, Germany, 2013), the 2013 Annual Meeting of the American Society of Clinical Oncology (Chicago, Illinois, USA, 2013) and the 12th International Conference on Malignant Lymphoma (Palazzo dei Congressi, Lugano, Switzerland, June 19-22).

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TABLES

Table 1A. Patients' characteristics and results of the univariate analyses of OS after five years (training dataset)

Variable	No. of patients N (%)	5-year survival (%)	Log rank, p	Univariable comparison	HR* (95% CI **)
All patients	**1948**	**80.3%**			
Sex	**1948**		<0.001		
Female	615 (31.6)	84.0			
Male	1333 (68.4)	78.6		*vs*. female	1.5 (1.2 – 1.9)
Time from diagnosis to study entry (years)	**1877**		0.004		
≤ 1	1187 (63.3)	84.1			
> 1	690 (36.7)	78.1		*vs.* ≤1	1.3 (1.1 – 1.6)
Age (years)	**1948**		0.003		
Median (range)	60.0 (30.0 - 81.0)				
≤ 60	1019 (52.3)	83.5			
> 60 and ≤ 65	511 (26.2)	76.7		*vs.* ≤60	1.5 (1.2 - 1.8)
> 65 and ≤ 70	275 (14.1)	78.9		*vs.* ≤60	1.3 (1.0 - 1.7)
> 70	143 (7.3)	73.2		*vs.* ≤60	1.4 (1.0 - 1.9)
B-symptoms	**1856**		<0.001		
No	1313 (70.7)	83.5			
Yes	543 (29.3)	72.3		*vs.* no	1.8 (1.5 – 2.2)

Binet stage	**1863**		<0.001		
A	793 (42.6)	89.5			
B	702 (37.7)	74.6			
C	368 (19.8)	70.1		B/C *vs.* A	3.0 (2.4 – 3.6)
Rai stage	**1863**		<0.001		
0	387 (20.8)	91.4			
I	342 (18.4)	82.5			
II	707 (37.9)	80.0			
III	161 (8.6)	64.4			
IV	266 (14.3)	72.0		0-I *vs.* II-IV	2.1 (1.7 - 2.6)
ECOG performance status	**1826**		<0.001		
0	1277 (69.9)	84.6			
1	533 (29.2)	70.3		0 *vs.* >0	2.1 (1.7 - 2.5)
2	16 (0.9)	62.5			
***IGHV* MS**	**1430**		<0.001		
Mutated	661 (46.2)	89.1			
Unmutated	769 (53.8)	70.9		*vs.* mutated	3.4 (2.7 – 4.3)
Deletion 6q	**779**		0.318		
No	746 (95.8)	84.0			
Yes	33 (4.2)	82.2		*vs.* no	1.3 (0.7 – 2.4)
Categories according hierarchical model[17]	**1557**		<0.001		
De(17p)	89 (5.7)	34.4		*vs.* del(13q)[¥]	5.9 (4.3 – 8.2)
Del(11q)[$]	250 (16.1)	75.4		*vs.* del(13q)[¥]	2.1 (1.6 – 2.8)

Trisomy 12[ß]	163 (10.5)	83.0		*vs.* del(13q)[ɣ]	1.1 (0.8 – 1.6)
Normal[#]	555 (35.6)	86.9		*vs.* del(13q)[ɣ]	0.8 (0.6 – 1.0)
Del(13q)[ɣ]	500 (32.1)	85.3			
Hemoglobin (g/dL)	**1863**		<0.001		
≤10.0	182 (9.8)	65.0		*vs.* >10.0	2.1 (1.6 – 2,7)
>10.0	1681 (90.2)	82.1			
Platelet count (x 10^3/µL)	**1859**		<0.001		
<100.0	262 (14.1)	70.0		*vs.* ≥100.0	1.9 (1.5 – 2.4)
≥100.0	1597 (85.9)	82.3			
Leukocyte count (x 10^3/µL)	**1866**		<0.001		
≤50.0	1069 (57.3)	86.0			
>50.0	797 (42.7)	75.6		*vs.* <50.0	2.4 (2.0 – 2.9)
Lymphocyte count (x 10^3/µL)	**1832**		<0.001		
≤50.0	1149 (62.7)	85.3			
>50.0	683 (37.3)	72.7		*vs.* ≤50.0	2.3 (1.9 – 2.8)
Absolute neutrophil count (x 10^3/µL)	**1698**		<0.001		
≤6.5	1284 (75.6)	83.2			
>6.5	414 (24.4)	75.2		*vs.* ≤6.5	1.6 (1.3 – 1.9)
LDH (U/L)	**1762**		<0.001		
≤210.0	1149 (65.2)	84.7			
>210.0	613 (34.8)	71.6		*vs.* ≤210.0	2.1 (1.7 – 2.5)

S-TK (U/L)	**1670**		<0.001		
≤10.0	801 (48.0)	91.7			
>10.0 and ≤24.0	450 (27.0)	78.6		*vs.* ≤10.0	3·1 (2.4 – 4.1)
>24.0	419 (25.0)	66.4		*vs.* ≤10.0	5·4 (4.2 – 7.0)
S-β_2m (mg/L)	**1676**		<0.001		
≤1.7	403 (24.0)	94.2			
>1.7 and ≤3.5	861 (51.4)	82.7		*vs.* ≤1.7	2.8 (2.0 – 3.9)
>3.5	412 (24.6)	67.9		*vs.* ≤1.7	6.5 (4.6 – 9.3)
ZAP-70 (%)	**502**		0.2		
≤20.0	310 (61.8)	73.5			
>20.0	192 (38.2)	68.6		*vs.* ≤20.0	1.2 (0.9 – 1.7)
CD38 (%)	**914**		0.8		
≤30.0	619 (67.7)	75.2			
>30.0	295 (32.3)	70.9		*vs.* ≤30.0	1.1 (0.9 – 1.4)

Table 1B. Patients' characteristics and results of the univariate analyses of OS after five years (Mayo validation dataset)

Variable	**No. of patients N (%)**	**5-year survival (%)**	**Log rank, p**	**Univariable comparison**	**Hazard ratio* (95% CI **)**
All patients	**676**				
Sex	**676**		0.2		
Female	223 (33.0)	92.9			
Male	453 (67.0)	87.1		*vs.* female	1.4 (0.9 - 2.2)
Age (years)	**676**		<0.001		
Median (range)	61.5 (32.0 -				

	89.0)				
≤60	314 (46.4)	94.3			
>60 and ≤65	116 (17.2)	91.6			
>65 and ≤70	102 (15.1)	88.3			
>70	144 (21.3)	77.0		>60 *vs.* ≤60	2.3 (1.4 - 3.7)
Rai stage	**676**		<0.001		
0	386 (57.1)	91.8			
I	230 (34.0)	85.0			
II	40 (5.9)	88.9		0 *vs.* I-II	1.6 (1.01 - 2.4)
III	7 (1.0)	51.4			
IV	13 (1.9)	92.3		0 *vs.* III-IV	4.5 (1.9 - 10.6)
ECOG performance status	**676**		<0.001		
0	635 (93.9)	89.8			
>0	41 (6.1)	70.6		0 *vs.* >0	1.8 (1.3 - 2.6)
***IGHV* MS**	**676**		<0.001		
Mutated	382 (56.5)	91.4			
Unmutated	294 (43.5)	85.1		*vs*. mutated	2.6 (1.7 - 4.0)
Categories according hierarchical model[17]	**676**		<0.001		
Del(17p)	30 (4.4)	39.7		*vs.* del(13q)[¥]	8.9 (4.5 - 17.5)
Del(11q)[$]	66 (9.8)	81.0		*vs.* del(13q)[¥]	2.6 (1.3 - 5.1)
Trisomy 12[$]	133 (19.7)	88.6		*vs.* del(13q)[¥]	1.7 (1.0 - 3.2)

Normal[#]	160 (23.7)	90.5		*vs.* del(13q)[ɣ]	1.2 (0.7 - 2.3)
Del(13q)[ɣ]	287 (42.5)	93.8			
S-TK (U/L)	**676**		0.01		
≤10.0	493 (72.9)	91.0			
>10.0	183 (27.1)	81.7		*vs.* ≤10.0	1.7 (1.1 - 2.8)
S-β_2m (mg/L)	**672**		< 0.001		
≤1.7	88 (13.1)	98.2			
>1.7 and ≤ 3.5	468 (69.6)	92.5		*vs.* ≤1.7	2.7 (0.9 - 8.8)
>3.5	116 (17.3)	65.5		*vs.* ≤1.7	11.6 (3.6 - 37.6)

* Hazard ratio

** Confidence interval

$ Not including deletion 17p

§ Not including deletion 17p and deletion 11q

No abnormalities according to the hierarchical model including deletion 17p, deletion 11q, trisomy 12 and deletion13q

ɣ Not including deletion 17p, deletion 11q and trisomy 12

Table 2. Results of the final Cox regression model and risk scores of independent factors based on 1223 patients with all parameters available (training dataset, score population)

Independent factor		HR* (95% CI**)	p	Risk score
Category according hierarchical model[17]	**Del(17p)**	**6.0** (4.2 – 8.6)	< 0.001	**6**
S-TK	**>10.0 U/L**	**2.1** (1.5 – 2.9)	< 0.001	**2**
S-β_2m	**>3.5 mg/L**	**2.3** (1.4 – 3.6)	0.001	**2**
S-β_2m	**>1.7 and ≤3.5 mg/L**	**1.7** (1.1 – 2.7)	0.01	**1**
***IGHV* MS**	**Unmutated**	**1.9** (1.5 – 2.5)	< 0.001	**1**
ECOG PS	**>0**	**1.7** (1.3 – 2.1)	< 0.001	**1**
Category according hierarchical model[17]	**Del(11q)**	**1.4** (1.03 – 2.0)	0.03	**1**
Sex	**Male**	**1.3** (1.01 - 1.6)	0.026	**1**
Age	**>60 years**	**1.3** (1.04 – 1.7)	0.045	**1**

* Hazard ratio

** Confidence interval

Table 3. Rates of 5-year OS according to the single risk scores and the risk groups of the prognostic index (training and validation dataset)

Risk Score	No. of patients N (%)	5-year overall survival, %	6-year overall survival, %
0	40 (3.3)	(all censored)	(all censored)
1	112 (9.2)	96.0	94.9
2	148 (12.1)	93.2	93.2
3	122 (10.0)	91.3	85.7
4	154 (12.6)	87.9	84.5
5	184 (15.0)	83.0	70.5
6	192 (15.7)	72.4	62.9
7	138 (11.3)	67.9	50.7
8	54 (4.4)	56.2	46.7
9	16 (1.3)	56.1	42.1
10	10 (0.8)	45.0	45.0
≥ 11	53 (4.3)	18.7	15.0

Risk group GCLLSG	Grouped risk score	No. of patients N (%)	5-year overall survival, %	6-year overall survival, %	HR* (95% CI**)
Low	**0 – 2**	300 (24.5)	95.2	94.8	
Intermediate	**3 – 5**	460 (37.6)	86.9	80.4	4.8 (2.9 – 8.0)
High	**6 – 10**	410 (33.5)	67.6	55.6	12.5 (7.7 – 20.5)
Very high	**11 – 14**	53 (4.3)	18.7	15.0	57.7 (33.0 – 101.2)
Risk group Mayo Clinic	**Grouped risk score**	**No. of patients N (%)**	**5-year overall survival, %**	**6-year overall survival, %**	**HR* (95% CI**)**
Low	**0 – 2**	226 (33.6)	95.2	92.1	
Intermediate	**3 – 5**	336 (49.9)	91.4	86.7	1.9 (1.03- 3.5)
High	**6 – 10**	95 (14.1)	71.7	69.1	5.5 (2.9 - 10.7)
Very high	**11 – 14**	16 (2.4)	13.6	13.6	28.9 (12.3 - 68.3)

* Hazard ratio

** Confidence interval

FIGURES

Figure Legends

Figure 1 shows the survival curves according to clinical staging in the training dataset.

Figure 2 shows time-to-event curves according to the prognostic index in the training dataset.

Figure 3 shows survival curves of Binet and Rai stages by prognostic index risk categories in the training dataset.

Figure 4 shows survival curves of genetic subgroups by prognostic index risk categories in the training dataset.

Figure 5 shows time-to-event curves according to the prognostic index in the validation dataset.

Figure 6 shows survival curves of Rai stages by prognostic index risk categories in the validation dataset.

Figure 7 shows survival curves of genetic subgroups by prognostic index risk categories in the validation dataset.

Figure 1. Survival curves according to clinical staging in the training dataset

Figure 1A and Figure 1B show overall survival (OS) of Binet and Rai stages within the training dataset (N = 1863, respectively)

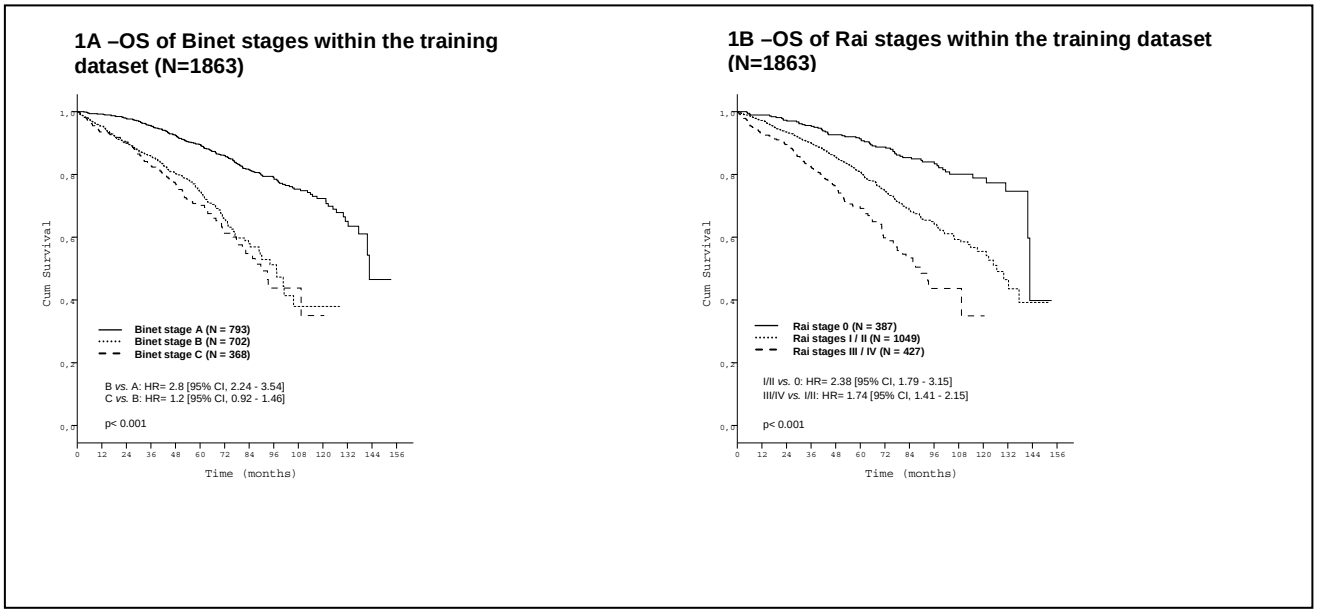


Figure 2. Time-to-event curves according to the prognostic index in the training dataset

Figure 2A shows overall survival (OS) by prognostic index risk categories in the training dataset (N=1223).

Figure 2B shows progression free survival (PFS) after first line treatment by prognostic index risk category in the training dataset (N=807).

Figure 2C shows treatment free survival (TFS) by prognostic index risk category of newly diagnosed patients initially managed with observation (watch-and-wait) among patients in the training dataset (N=416).

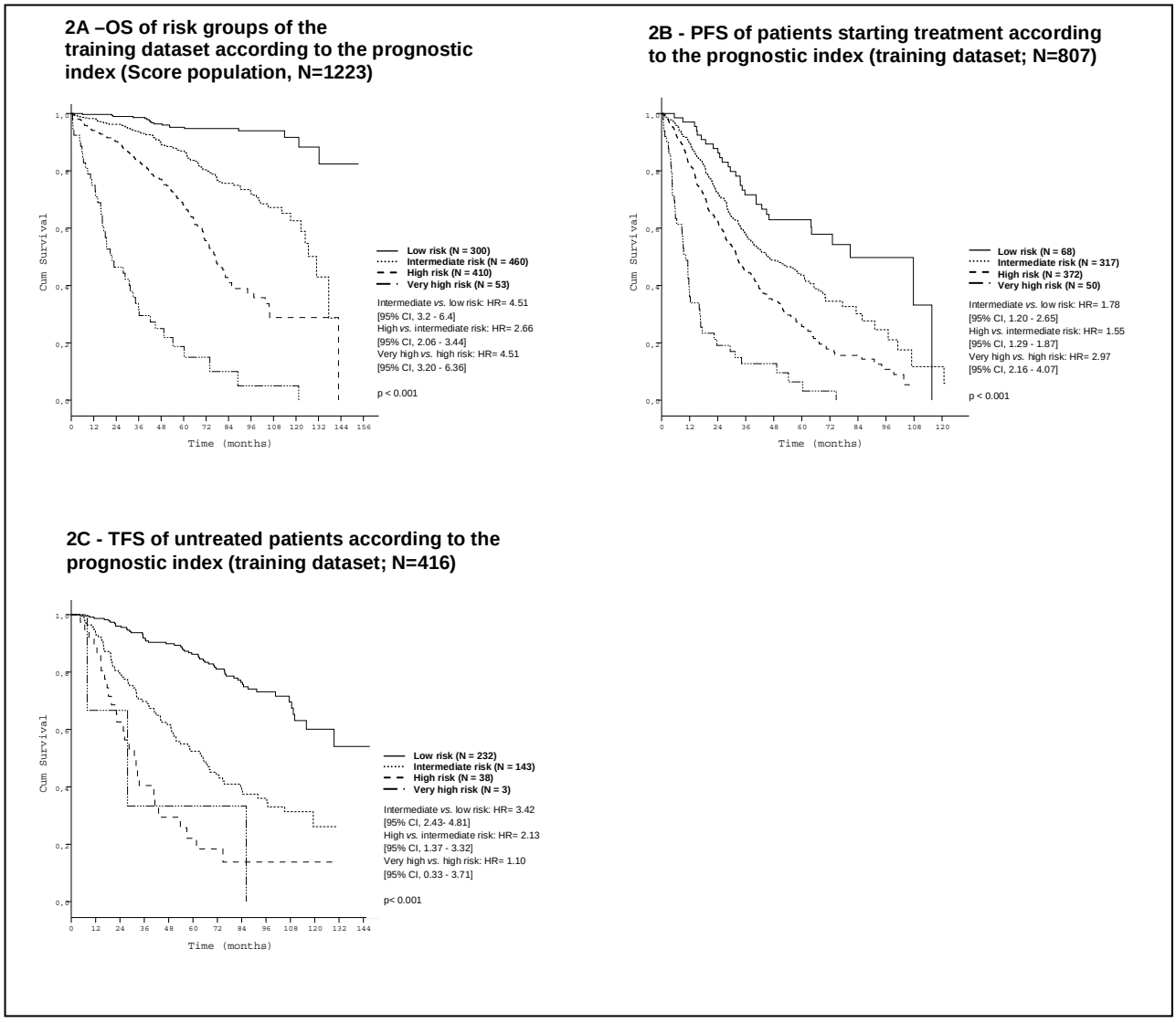


Figure 3. Survival curves of Binet and Rai stages by prognostic index risk categories in the training dataset

Figures 3A - 3C and Figures 3D - 3F show the overall survival (OS) of Binet and Rai stages by prognostic index risk categories in the training dataset.

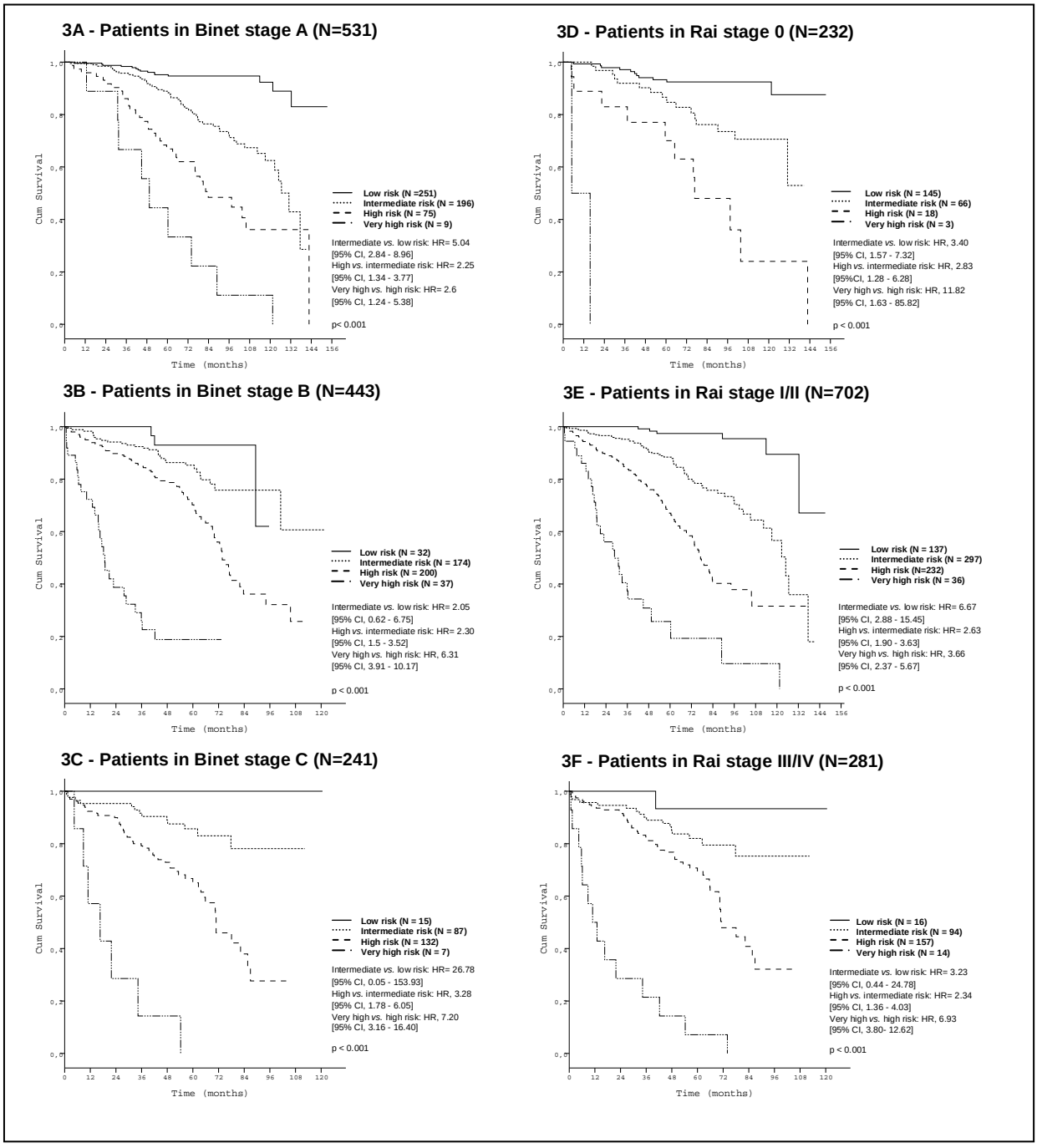


Figure 4. Survival curves of genetic subgroups by prognostic index risk categories in the training dataset

Figure 4A shows overall survival (OS) of *IGHV* unmutated patients by prognostic index risk category in the training dataset (N=645).

Figure 4B shows overall survival (OS) of 17p deleted patients by prognostic index risk categories in the training dataset (N=74).

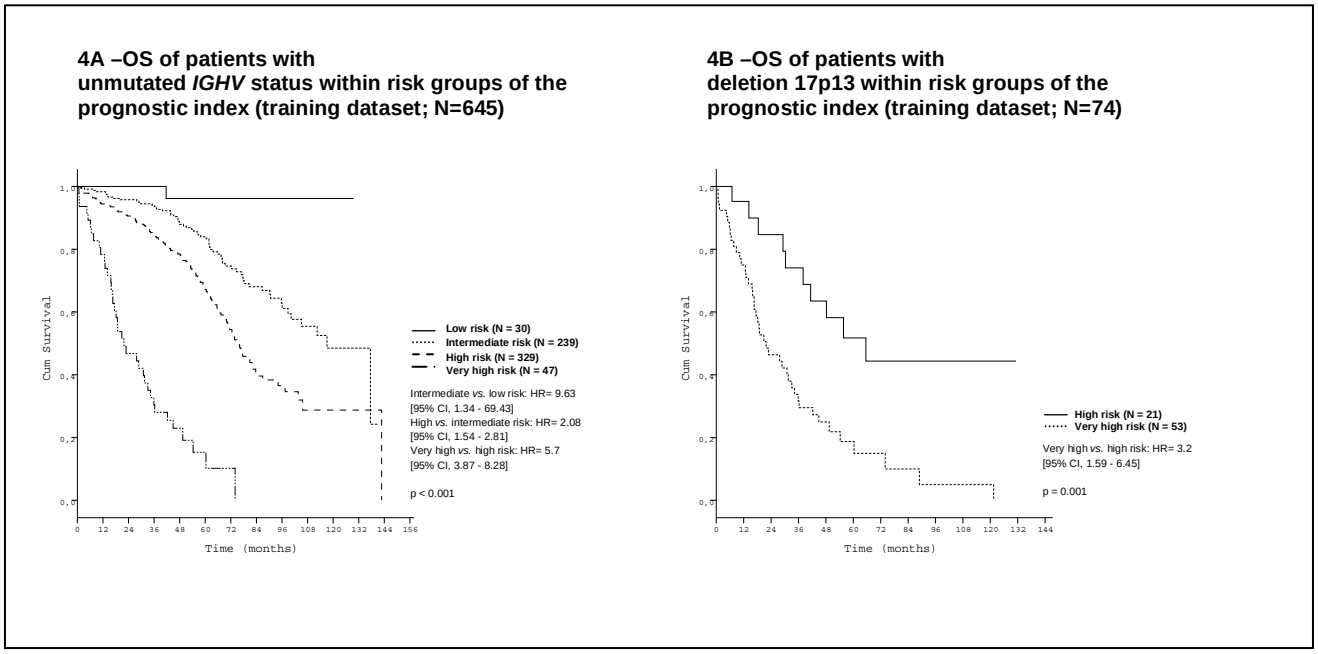


Figure 5. Time-to-event curves according to the prognostic index in the validation dataset

Figure 5A shows overall survival (OS) by prognostic index risk categories in the validation dataset (N=74).

Figure 5B shows treatment free survival (TFS) by prognostic index risk categories in the validation dataset (N=673).

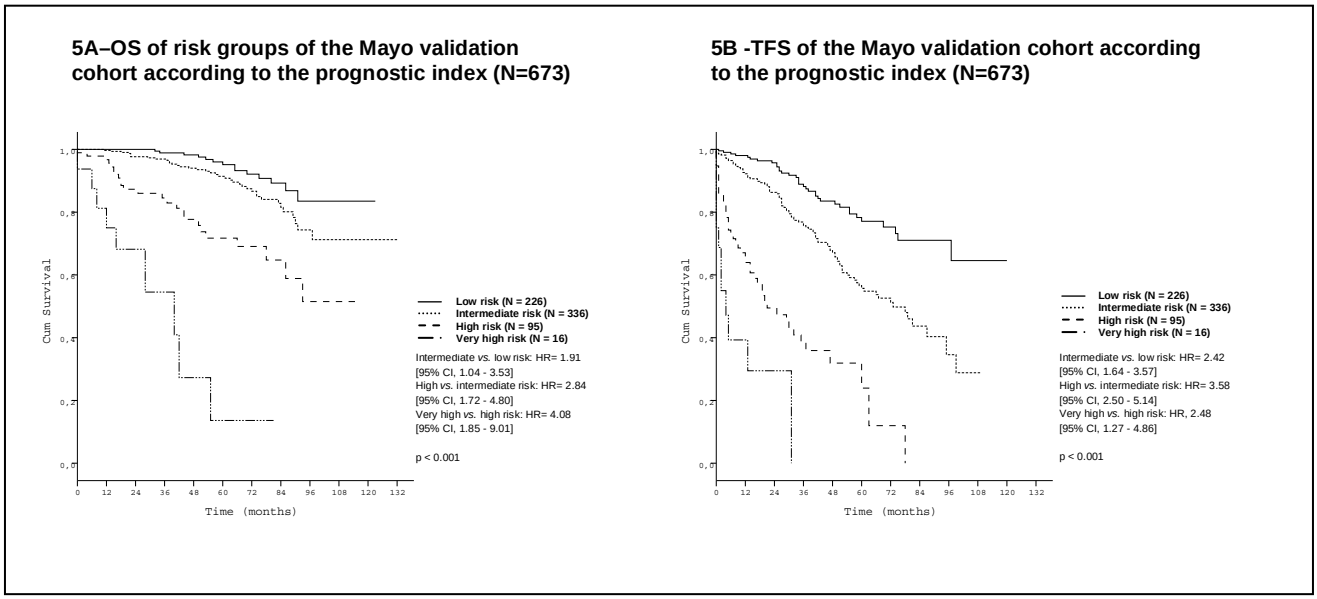


Figure 6. Survival curves of Rai stages by prognostic index risk categories in the validation dataset

Figures 6A - 6C show the overall survival (OS) of Rai stages by prognostic index risk categories in the validation dataset.

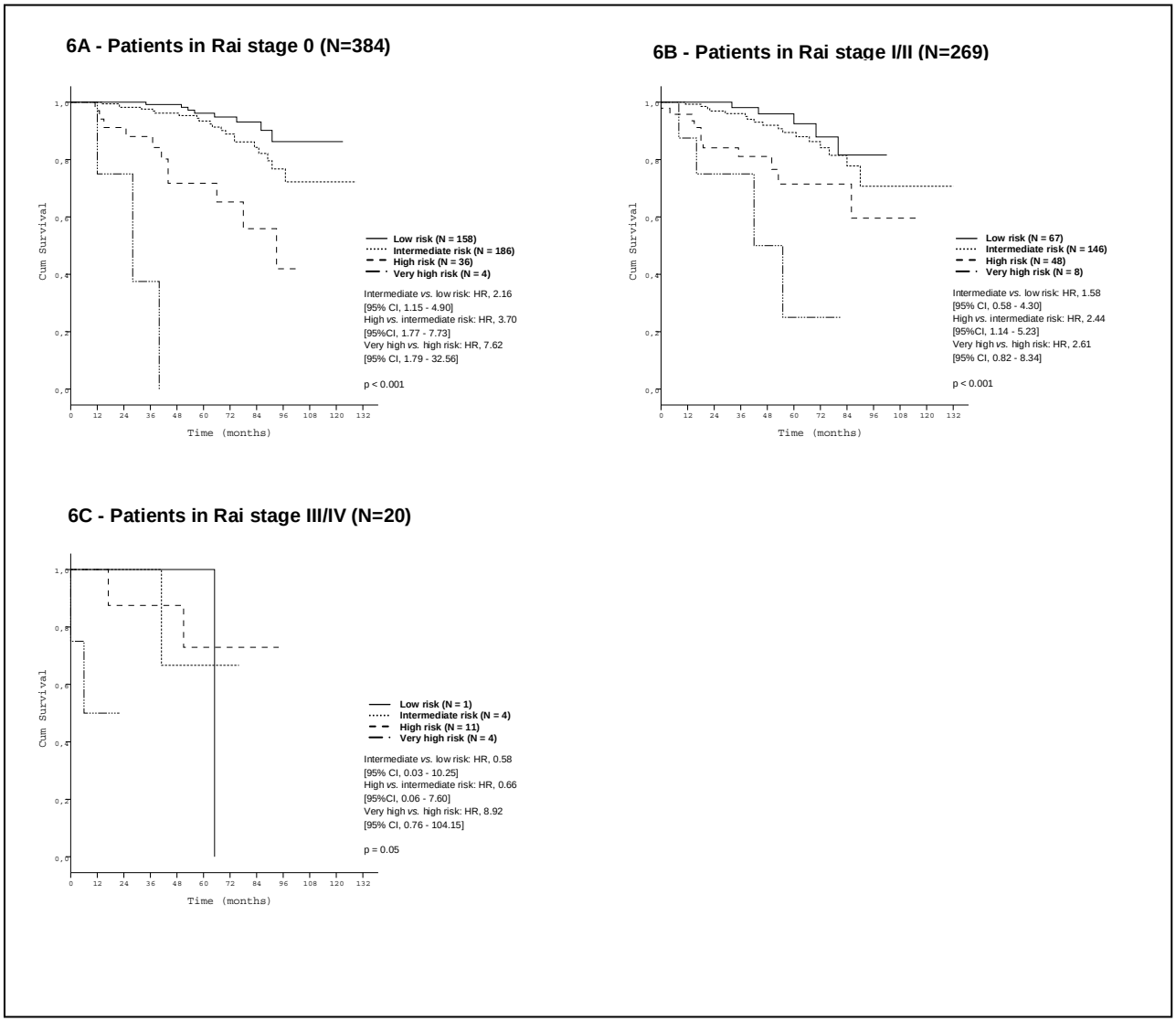
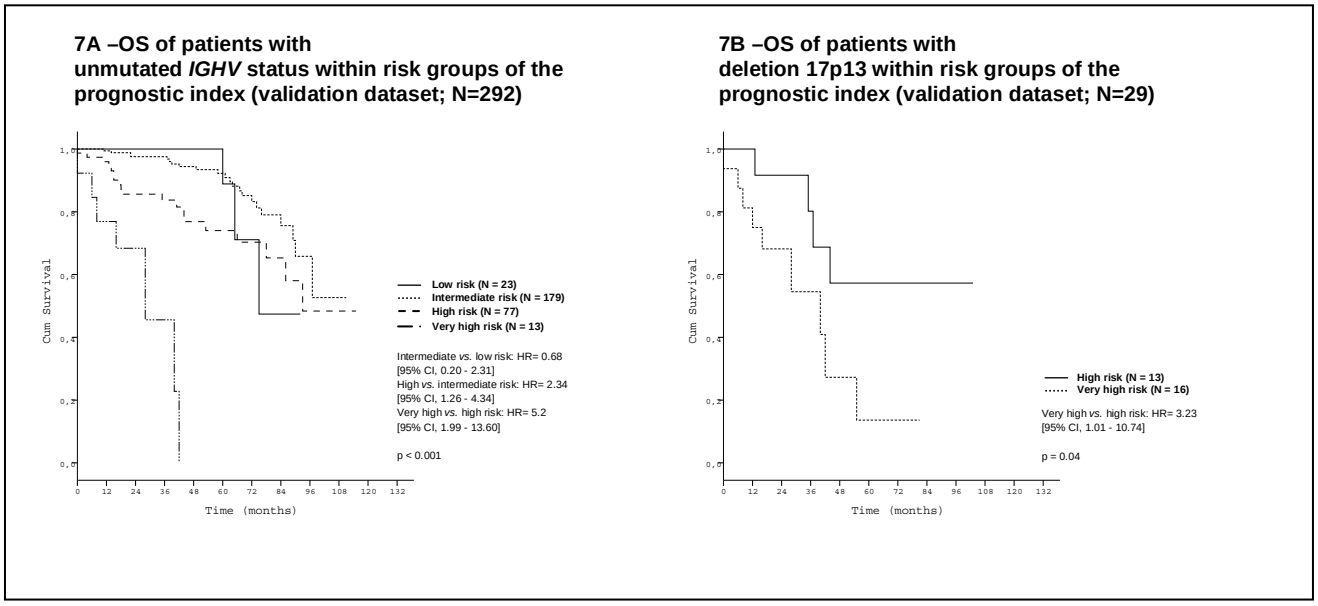


Figure 7. Survival curves of genetic subgroups by prognostic index risk categories in the validation dataset

Figure 7A shows overall survival (OS) of *IGHV* unmutated patients by prognostic index risk category in the validation dataset (N=292).

Figure 7B shows overall survival (OS) of 17p deleted patients by prognostic index risk categories in the validation dataset (N=29).



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Development of a comprehensive prognostic index for patients with chronic lymphocytic leukemia

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