Chronic Lymphocytic Leukemia: which prognostic factor to choose?

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Background

Chronic Lymphocytic Leukemia (CLL) has an extremely variable clinical course with overall survival time ranging from months to decades. For some patients, the disease runs an indolent clinical course and life expectancy is not shortened; for others, the disease is aggressive, progresses rapidly and survival after diagnosis is decreased to 2-3 years. Therefore it is very important to identify factors that can predict poor prognostic and also identify patients who will benefit from intense therapy in an early stage. These two different groups in terms of overall survival and clinical characteristics were classified for a long time on Binet Stage and more recently on the IgVH mutational status that seems to be one of the most robust biological prognostic factors. However, this costly analysis is very laborious and time-consuming. Therefore, many surrogate markers have been investigated. Finally, among all these factors, one question remains: which prognostic factor to choose?

Methods

We compared the most commonly used prognostic factors (Binet Stage, IgVH mutational status, Zap-70, CD38 and LPL expression) in a cohort of 108 patients with a median follow-up of 82 months to evaluate their association with overall survival (OS) and treatment-free survival (TFS). Flow cytometry (FC) and quantitative PCR (qPCR) on purified CD19+ cells were used. Association of surrogate markers with IgVH mutational status (using χ^2 Pearson and Cramer's V statistic), optimal cut-off values of Zap-70, LPL and CD38 that best distinguished between mutated and unmutated cases (evaluated with ROC curve analysis), power of prognostic marker at one and two years after diagnosis (evaluated with time-dependent ROC curves), OS and TFS distributions (using Kaplan-Meier estimates and the log-rank test) and finally the impact of the different prognostic factors on TFS and/or OS (evaluated with univariate and multivariate Cox regression analysis with binarized data) were performed.

	n	IgVH Unmut.	%	IgVH Mut.	%	*P*	—	*Cramer's V Statistic*
Patients	105	51	**49**	54	**51**	N.S.	1.83	0,13
Male	62	34	**55**	28	**45**			
Female	43	17	**40**	26	**60**			
Binet Stage A	72	29	**40**	43	**60**	0.004	11.27	0.33
Binet Stage B	20	13	**65**	7	**35**			
Binet Stage C	10	9	**90**	1	**10**			
Mutational status [a]								
IgVH - Unmutated	-	-	-	-	-	-	-	-
IgVH - Mutated	-	-	-	-	-	-	-	-
Zap-70 (Real time RT-PCR) [b]						<0.0001	50.95	0.72
>115 (positive)	54	45	**83**	9	**17**			
<115 (negative)	51	6	**12**	45	**88**			
LPL (Real time RT-PCR) [b]						<0.0001	24.08	0.50
>6 (positive)	49	38	**78**	11	**22**			
<6 (negative)	56	14	**25**	42	**75**			
Zap-70 (flow cytometry) [c]						<0.0001	26.32	0.56
>20% (positive)	42	33	**79**	9	**21**			
<20% (negative)	49	11	**22**	38	**78**			
CD38 (flow cytometry) [b]						0.002	10.07	0.36
>7% (positive)	53	32	**60**	21	**40**			
<7% (negative)	38	9	**24**	29	**76**			
Patients requiring no treatment	46	14	**30**	32	**70**	<0.0001	15.94	0.40
Patients requiring treatment	52	35	**67**	17	**33**			
Patients still alive	83	36	**43**	47	**57**	0.004	8.47	0.31
Patients died during the study	15	13	**87**	2	**13**			

[a] Mutational status is based on a 98% cut-off value.
[b] The cut-off determined using ROC curve analysis is expressed in fold of target gene expression in a calibrator cell line
[c] The cut-off of 20% of CD19+ cells that express Zap-70 by flow cytometry

Table 1. Cross-tabulations of prognostic markers vs IgVH mutational status

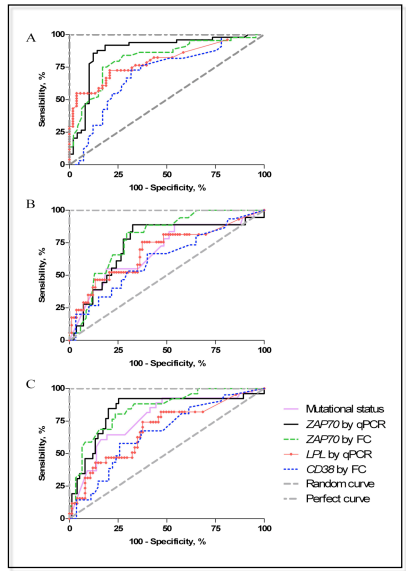

Fig 1. ROC curve analysis and ROC time-dependent curves

Results

All prognostic factors tested were associated with IgVH mutational status but Zap-70 measured by qPCR [P<0.0001] was characterised by the higher Cramer's V statistic (0.72) indicating a very strong relation (Table 1). This method also presents 87.8% sensitivity, 85.7% specificity, 87.5% positive predictive value and 86% negative predictive value (Fig. 1A). The concordance rate between Zap-70 and IgVH mutational status were largely higher than other factors (78% and 86% respectively for Zap-70 by FC and qPCR). All prognostic factors were significant TFS predictor (regarding log-rank test and univariate Cox regression) but only IgVH mutational status [*P=0.0034*] and Zap-70 [by both methods: FC, *P=0.0006*; qPCR, *P=0.0021*] were significant OS predictors. For example, Zap-70-positive patients had a significantly shorter median TFS (24 months) than Zap-70-negative patients (157 months) (Fig 2). Moreover, in case of discordance with IgVH mutational status, only Zap-70 by qPCR was associated with TFS [*P=0.0395*].

	Association with MS	Strength of association with MS	AUC prediction of MS	Concordance with MS	Assoc. with TFS	Assoc. with OS	TFS in case of discordance with MS	Univariate Cox predictor of TFS	Univariate Cox predictor of OS	Multivariate Cox predictor of TFS	1 year AUC predictor of TFS	2 years AUC predictor of TFS
Mut.status(MS)	-	-	-	-	S	S	-	S	S	NS	70%	77%
Zap-70(qPCR)	S	very strong	89%	86%	S	S	S	S	S	S	74%	83%
Zap-70(FC)	S	strong	85%	78%	S	S	NS	S	S	S	79%	84%
LPL (qPCR)	S	strong	76%	75%	S	NS	NS	S	NS	NS	69%	69%
CD38(FC)	S	substantial	70%	67%	S	NS	NS	S	NS	NS	63%	66%

S: significant; NS: non significant

Table 2. Summary of all analysis

Multivariate Cox regression including Zap-70 (by qPCR or by FC), LPL by qPCR, mutational status and CD38 expression indicated also that Zap-70 [by qPCR:*P=0.038*, by FC:*P=0.005*] was more powerful to predict TFS than the classical mutational status and the other markers tested. Time-dependent ROC curves were also generated to evaluate the power of all markers tested at one and two years after diagnosis: the Area Under the Curve of Zap-70 expression (by both methods) (AUC) was higher than the other prognostic factors including IgVH mutational status (Fig. 1B, 1C). For example, 2 years AUC was 0.83 for Zap-70 by qPCR, 0.84 for Zap-70 by FC while this value was 0.77, 0.69, 0.66 respectively for IgVH mutational status, LPL and CD38 expression.

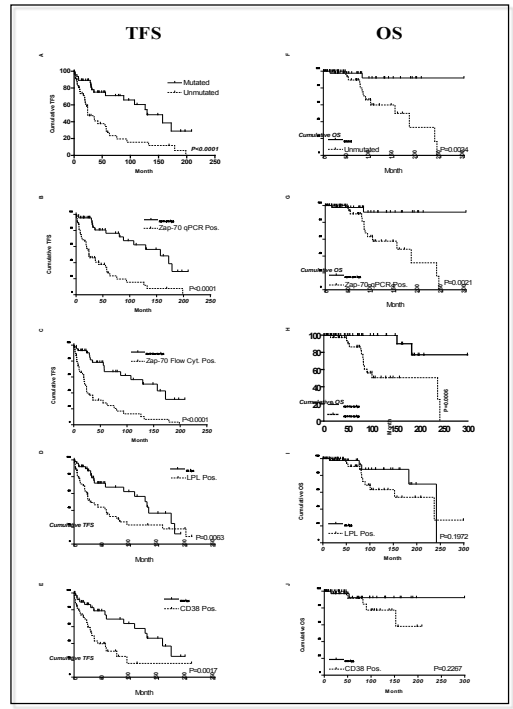

Fig 2. Kaplan-Meier survival curves for TFS and OS

Conclusions

Regarding all these analysis (Table 2), we conclude that Zap-70 is the most powerful prognostic factor and the best surrogate of IgVH mutational status among all factors tested. The choice of the method to measure Zap-70 is more complicated but the qPCR method is more accurate, can offset FC limitations, is strongly associated with IgVH mutational status, prevalent on this status in case of discordance, and in case of discordance with Zap-70 by FC, Zap-70 by qPCR shows a clear trend to be prevalent. Therefore we recommend the use of Zap-70 measured by qPCR as prognostic factor.