Zap-70 mRNA expression quantified in B cells by real time PCR is a powerful prognostic factor in Chronic Lymphocytic Leukemia

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Introduction

Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease with respect to prognosis and clinical outcomes. Two different groups in term of overall survival and clinical characteristics are now classified on the IgVH mutational status. However, this costly analysis is very laborious. Therefore surrogate markers have been investigated.

Methods

We developed a standardised and quantitative PCR (qPCR) method to measure Zap-70 mRNA (Zeta-associated protein 70) expression in purified CD19+ cells. The comparison of this method with others (Zap-70 and CD38 by flow cytometry, and lipoprotein lipase (LPL) mRNA by qPCR) was performed in a cohort of 108 patients with a median follow-up of 82 months (range 8-299) to evaluate their association with IgVH mutational status, overall survival (OS) and treatment-free survival (TFS).

Results

ASSOCIATION BETWEEN ZAP-70 BY qPCR AND MUTATIONAL STATUS

The association between Zap-70 by qPCR and IgVH mutational status was clearly significant [$\chi^2(1)=50.95$;P<0.0001] and characterised by a Cramer's V statistic of 0.72 indicating a very strong relation. The other prognostic factor tested were also significant but their association with mutational status was characterised as substantial to good as indicated by the Cramer's V value (Table 1). Concordance rates with mutational status were 86%, 78%, 75% and 67% respectively for Zap-70 by qPCR, Zap-70 by FC, LPL by qPCR and CD38 by FC. To estimate the power of these different markers and to predict correctly the mutational status, sensitivity, specificity, positive (PPV) and negative (NPV) predictive values were evaluated. Zap-70 expression by qPCR showed 87.8% sensitivity, 85.7% specificity,87.5% PPV and 86% NPV. These performance indices were clearly better than the other markers.

	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

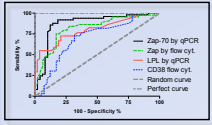

Fig. 1 Curve analysis of the different prognostic factors vs. mutational status

PROGNOSTIC VALUES OF ZAP-70 EXPRESSION BY qPCR

Zap-70 expression was significantly associated with OS [P=0,0021] and TFS [P< 0,0001] (Fig. 2). Zap-70-positive patients had a significantly shorter median TFS (24 months) than Zap-70-negative patients (157 months). Moreover, Zap-70 measured by qPCR have a better prognostic power than IgVH mutational status and the other prognostic markers tested (Table 2).

	Association with MS	Strength of association with MS	AUC: prediction of MS	Concordance with mutational status	Associat ion with TFS	Associat ion with OS	TFS in case of disconcordance with MS	Univariate Cox predictor of TFS	Univariate Cox predictor of OS	Multivariate Cox predictor of TFS	5 year AUC predictor of TFS	2 years AUC predictor of TFS
Mutational status (MS)	-	-	-	-	S	S	-	-	-	NS	78%	77%
Zap-70by qPCR	S	very strong	89%	86%	S	S	S	S	S	S	74%	83%
Zap-70by FC	S	strong	85%	78%	S	S	NS	S	S	S	79%	84%
LPL by qPCR	S	strong	76%	75%	S	NS	NS	S	NS	NS	69%	69%
CD38by FC	S	substancial	70%	67%	S	NS	NS	S	NS	NS	63%	66%

S=significant; NS: non significant

Table 2. Summarazing table

Moreover, we evaluated the area under the ROC curve (AUC) and applied a non-parametric statistical test to compare them (Fig. 1). The AUC measure reflects the probability of correct discrimination between actually positive and actually negative findings.

Conclusions

Zap-70 mRNA quantification in B cells by real time PCR is a strong surrogate marker of IgVH mutational status and is highly associated with TFS and OS. Therefore, we think this user-friendly and standardised technique could be used in routine laboratory to better evaluate the outcome of CLL patients and the need treatment.

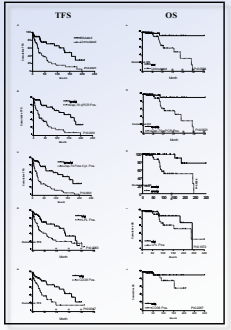

Fig 2. Kaplan-Meier survival curves for TFS and OS