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Predictive value of β2-microglobulin (β2-m) levels in chronic lymphocytic leukemia since Binet A stages

We read with interest the study by Rossi and coworkers, reporting CD49d expression as risk factor of treatment free survival (TFS) in Binet A CLL patients.[1] In this paper, a close association between CD49d and CD38, LDH and β2-m is also described. We would like to add further information about the prognostic power of β2-m. It is generally believed that β2-m is released constitutively by CLL cells and that its level approximately correlates with tumor mass.[2] Based on these premises the predictive value of β2-m serum concentration could vary in the course of the disease and be relatively low in the early disease stages, when tumor mass is low, irrespective of the subsequent clinical outcome. Therefore, β2-m determination could exhibit a lower predictive power particularly at the early disease stages compared to the newer biological markers, such as IgVH gene status, ZAP-70 and CD38, which represent intrinsic cell features that can be determined since the earliest disease stages and never (IgVH) or rarely (ZAP-70 and CD38) change in the course of the disease.[3]

In order to explore this issue, we have measured β2-m value in 222 Binet stage A patients at diagnosis. IgVH gene status and CD38 expression were also determined in all cases studied. Unlike β2-m, which was measured at diagnosis, these markers were determined in the course of the disease when marker determinations became available. This approach, although irrelevant for the IgVH gene status, may introduce some, albeit minor, biases for CD38 for the reasons alluded to above. The median β2-m value was 2 mg/dL (range 0.4-19). ROC analysis determined that the cut-off value capable of discriminating between patients whose disease progressed and required treatment from those with stable disease was 2.4 mg/dL (AUC:0.67, p=0.005). Accordingly 149/222 patients (67%) were β2-m^{neg} and 73/222 (33%) as β2-m^{pos}. Overall, the results did not substantially change when arbitrary cut offs used by other authors[4-7] were employed.

The patients' features are summarized in Table 1. β2-m levels overlap with CD38 expression in 128/219 cases (63%) [β2-m^{pos}/CD38≥30% cases: 23/55 (41.8%), β2-m^{neg}/CD38<30% cases: 115/164 (70.1%)], while β-m levels overlap with IgVH status in 125/195 cases (64.1%) [β2-m^{pos}/IgVHunmutated cases: 29/62 (46.8%), β2-m^{neg}/IgVHmutated: 96/133 (72.2%)]. Finally, the concordance between CD38 expression and IgVH mutational status was 77.6% (149/192 cases) [IgVHunmutated/CD38≥30% cases: 35/52 (67.3%), IgVHmutated/CD38<30% cases: 114/140 (81.4%)].

After a median follow-up of 3.5 years, 55 of 222 Binet stage A (25%) required treatment. β2-m^{neg} cases showed a significantly longer TFS than β2-m^{pos} cases; in particular the projected median TFS was 5.3 years for β2-m^{pos} versus not reached for β2-m^{neg} (Figure 1A). TFS represented a reliable measure of disease progression since all centers agreed to follow NCI guidelines for treatment start.

In order to ascertain whether β2-m identifies a patient subset of those with good prognostic markers, we calculated TFS of both CD38<30% and IgVHmutated CLL cases grouped according to the β2-m expression. β2-m^{pos} CD38<30% cases exhibited a TFS which was significantly lower than that of β2-m^{neg} CD38<30% cases (3.5-years TFS probability: β2-m^{neg} *vs.* β2-m^{pos} 91% *vs.* 83%; p=0.05). However, these differences were not seen in the IgVHmutated cases (3.5-years TFS probability: β2-m^{neg} *vs.* β2-m^{pos} 89% *vs.* 84%; p=ns).

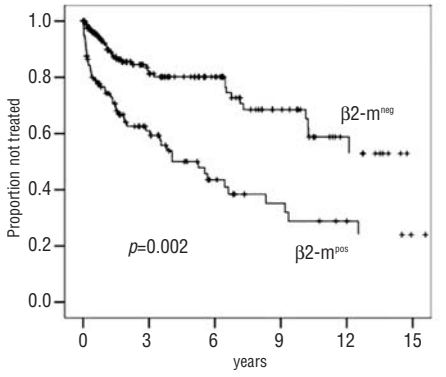
At Cox univariate analysis, β2-m^{pos} (HR:2.3, p=0.003), CD38>30% (HR:3.9, p<0.0001) and IgVHunmutated (HR:3.2, p<0.0001) showed a statistically significant impact on TFS. At Cox multivariate analysis, all the three markers maintained an independent prognostic impact (β2-m^{pos}, HR:1.8, p=0.047; CD38>30%, HR:2.0, p=0.03; IgVHunmutated, HR:2.7, p=0.022). When a scoring system in which one point was assigned to each unfavorable prognostic marker was utilized, the risk of an early treatment was highest (Figure 1B) in patients presenting all the three adverse prognostic markers. Cases with two, one or none of the unfavorable prognostic factors showed lower risk for an early treatment (Figure 1C).

Collectively, this study shows that β2-m levels represent valuable predictors in early CLL stages, when the neoplastic cell burden is low. This finding raises a number of questions regarding the mechanisms governing the β2-m levels. This molecule is constantly shedded[8]

Table 1. Comparisons of clinical and laboratory features among chronic lymphocytic leukemia patients devised according to β2-m expression.

	All patients	β2-m <2.4 mg/d	β2-m ≥2.4 mg/dL	p
N. of patients	222	149 (67)	73 (33)	
Age (years)				
≤65	124 (56)	94 (63)	30 (41)	0.002
>65	98 (44)	55 (37)	43 (59)	
Gender				
Female	82 (37)	60 (40)	22 (30)	0.14
Male	140 (63)	99 (60)	51 (70)	
IgVH mutational status (n=195)				
Mutated	133 (68)	96 (74)	37 (56)	0.014
Germline	62 (32)	33 (26)	29 (44)	
CD38 expression (n=219)				
<30%	164 (75)	115 (78)	42 (58)	0.02
≥30%	55 (25)	32 (22)	30 (42)	
Therapy				
no	167 (75)	123 (83)	44 (60)	<0.0001
yes	55 (25)	26 (17)	29 (40)	

A



B

Risk categories	Univariate analysis HR (95% C.I., *p* value)
No factor	1
One factor	1,5 (0.7-3.4, *p*=ns)
Two factor	5.0 (2.5-10.2, *p*<0.0001)
Three factors	15.4 (7.3-32.5, *p*<0.0001)

C

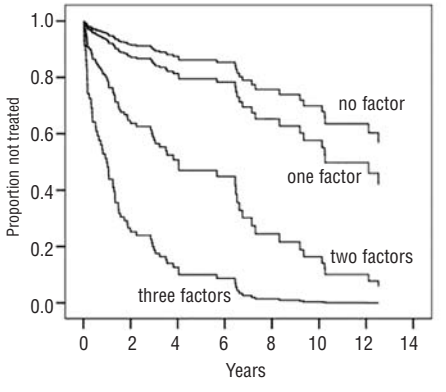


Figure 1. (A) **Kaplan–Meier estimates of treatment-free survival (TFS). Comparison between β2-m^neg patients versus β2-m^pos patients.** (B) **Cox univariate analysis for the predictive value of marker combinations for the risk categories.** (C) **Cox derived estimated TTT curves according to the combination of the three prognostic factors.**

by lymphocytes and it is expected that its levels steadily increase together with the progressive expansion of the leukemic clone suggesting a close correlation between stage (which measures tumor burden) and β2-m levels. Although a correlation with disease stage likely exists, there was a substantial proportion of patients with high β2-m levels already at Binet A stage (low tumor burden). Possibly, CLL cells from these patients are more activated *in vivo* and shed more abundant β2-m. Taken all the above into consideration, the data indicate that the role of β2-m as a prognostic tool should be re-evaluated possibly in prospective studies involving large patient cohorts.

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Key words: β2-microglobulin, CD38, IgVH mutational status, CLL, prognosis.

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Detection of continuous erythropoietin receptor activator in blood and urine in anti-doping control

Anti-doping control of erythropoietin (Epo) relies on the differentiation by isoelectric profile of natural endogenous hormone from the recombinant hormone used for doping. The first and second generations of recombinant Epo were detectable in urine.[1,2] The third generation, Continuous Erythropoietin Receptor Activator (CERA), was obtained by linking a methoxy polyethylene glycol to epoetin β, a first generation rHuEPO, resulting in significantly greater stability in blood. CERA, approved in Europe in July 2007, was