A high proportion of cells carrying trisomy 12 is associated with a worse outcome in patients with chronic lymphocytic leukemia

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Abstract:

The prognosis of CLL patients displaying trisomy 12 (+12) remains unclear.

In this study, we analyzed the influence of the proportion of cells with +12, and other clinical and biologic factors, in time to first therapy (TTFT) and overall survival (OS), in 289 patients diagnosed with CLL carrying +12. Median OS was 129 months. 174 patients (60.2 %) presented +12 in <60% of cells. TTFT and OS for this subgroup were longer than for the subgroup with +12 in ≥ 60% of cells, with a median TTFT of 49 months (CI95%, 39-58) *vs* 30 months (CI95%, 22-38) (P=0.001); and a median OS of 159 months (CI95%, 119-182), *vs* 96 months (CI95%, 58-134) (P=0.015). Other factors associated with a shorter TTFT were: Binet stage, B symptoms, lymphadenopathy, splenomegaly, high lymphocyte count, 11q-, high $\beta_2$microglobulin, and high LDH. In the multivariate analysis, clinical stage, +12 in ≥60% of cells, high lymphocyte count, B symptoms and 11q- in addition, resulted of significance in predicting shorter TTFT. Significant variables for OS were: Binet stage, lymphadenopathy, splenomegaly, high LDH, high $\beta_2$microglobulin, 11q-, and CD38. In the multivariate analysis, only Binet stage, 11q-, and high β2microglobulin significantly predicted shorter OS. CLL with +12 entails a heterogeneous group with intermediate prognosis. However, a high proportion of cells carrying +12 separates a subgroup of patients with poor outcome.

Introduction

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, with survival times ranging from months to decades [1, 2, 3]. Clonal genomic aberrations can be identified in approximately 80% of CLL patients by fluorescent *in situ* hybridization (FISH), and they constitute one of the most important predictors of disease progression and survival. The most common recurrent chromosomal abnormalities include: 13q deletion (13q-); 11q deletion (11q-); trisomy 12 (+12); and 17p deletion (17p-), defining five prognostic categories with survival times ranging from 32 months in patients with 17p- to 133 months in patients with 13q- as the sole abnormality [4]. Recently, a new prognostic scoring system which separates CLL patients into four prognostic risk groups has been published: high-risk, harboring *TP53* and/or *BIRC3* abnormalities (10-year OS: 29%); intermediate-risk, harboring *NOTCH1* and/or *SF3B1* mutations and/or del(11q22-q23) (10-year OS: 37%); low-risk, harboring trisomy 12 or a normal genetics (10-year OS: 57%); and very low-risk, harboring del(13q) only, whose 10-year OS (69.3%) did not significantly differ from a matched general population [5].

Trisomy 12 is the third most frequent cytogenetic aberration in CLL, occurring in up to 15-20%. It often appears as the unique cytogenetic alteration (40-70% of cases with +12), although it can be associated with other chromosomal aberration [6].

The critical genes involved in this aberration remain unknown [7] and it has been associated with an atypical morphology or immunophenotype [8]. CLL patients with this aberration have been classically considered to have an intermediate prognosis [4], although evidence from prospective trials suggests that overall survival is favorable despite progression-free survival may be shorter [7, 9] .

CLL patients with +12 rarely show TP53 mutations and rarely acquire these over time, finding that may partly explain the benign course after treatment [5, 10]. However, the

presence of *NOTCH-1* mutations can be identified in 30-40% of patients carrying +12, and it confers a worse disease outcome in this subset of patients [11, 12, 13, 14].

Several studies demonstrated that the presence of a specific cytogenetic abnormality is not sufficient to identify homogeneous subgroups of patients. Thus, a high proportion of cells carrying 13q14-, or large deletions including RB1 gene are associated with a worse outcome [15, 16, 17, 18]. Similar results have been observed regarding 11q-, with a negative prognostic impact when 11q- involves the majority of CLL clone [19], and regarding 17p-, with a worse clinical outcome when a higher percentage of deleted cells is present [20]. Therefore, a multicenter analysis including 289 patients diagnosed with CLL harboring +12 was carried out. We described and compared the clinical and biological characteristics of these patients, and found that a high number of +12 lymphocytes has a bad impact in the clinical outcome.

Patients and methods

Patients

An electronic database containing information from 2,561 patients diagnosed with CLL from 25 Spanish institutions was retrospectively screened. The diagnosis was based according to the World Health Organization Classification of Tumors [21] and International Workshop on CLL guidelines [22]. All (289) cases carrying +12 detected in the routine FISH analysis were selected. Clinical information recorded at diagnosis included age, Binet stage, and physical examination. Analytical parameters included absolute white blood cell and lymphocyte counts, serum lactate dehydrogenase (LDH) and serum beta2-microglobulin (B2M) concentrations. Prognostic factors such as CD38, ZAP70 expression, and mutational status of immunoglobulin heavy chain (IGHV) were collected when available. The study was approved by the local ethics committee and all individuals provided their informed consent.

FISH analysis

Interphase FISH was performed on peripheral blood samples at the time of diagnosis using commercially available probes for the following regions: 11q22/ATM, 12q13, 13q14 and 17p13/TP53 (Vysis/Abbott Co, Downers Grove, IL, USA). Methods for FISH analysis are described elsewhere [23]. Dual color FISH using differently labeled control probes was performed, and signal screening was carried out on at least 200 cells with well delineated signals. Hybridization was repeated in those slides with less than 80% cells showing two control-probe signals. The sensitivity limit for the detection of +12 and deletions were >5%, and >10% interphase cells with three signals and one signal respectively.

Statistical analysis

Statistical analysis was performed using the SPSS 21.0 software package (SPSS, Chicago, IL, USA). The cut-off point for percentage of +12 was selected by dividing the variable into deciles and selecting the most efficient cut-point. The Fischer´s exact test and the Chi-squared test were used to determine the relationship between categorical variables. Quantitative variables were compared by using the Student-t test and the Mann–Whitney U test.

OS was calculated from the time of diagnosis to death or last follow-up visit. TTFT was calculated as the interval between diagnosis and the start of first line treatment. OS and TTFT were estimated by the Kaplan-Meier method and assessed by the log-rank test. Univariate and multivariate analysis were performed using Cox regression method. Statistical significance was defined as $P < 0.05$.

Results

Patient characteristics

FISH detected +12 in 355 patients (13.9%) of the 2,461 patients initially included in the study. The final analysis was limited to 289 cases, after excluding monoclonal B-cell lymphocytosis, cases that acquired +12 as clonal evolution, or with inadequate follow-up. Median age was 68 years old (range, 22-88 years). A hundred and seventy-eight patients were male (61.6%). At the time of diagnosis, most cases were classified as stage A (68.9%), while only 5.2% were in stage C according to Binet classification [3]. Median white blood cell (WBC) count was $19x10^9$/L, and only 16.3% of patients presented with >30 $x10^9$/L lymphocytes. Splenomegaly, hepatomegaly, and lymph node involvement were present in 15.5%, 5.7%, and 54.1%, respectively. Regarding *IGHV* mutation status, 53.8% of the patients were classified as unmutated. CD38 and ZAP-70 were positive in 37.4%, and 55.8% of the patients, respectively. Multiple genetic abnormalities detected by FISH were present in 56 cases including: 11q- (3.9%), 13q- (17%), and 17p- (6.1%). 78.2% of patients were alive at the time of analysis, and median follow up was 41 months (range 1-197 months).

The impact of proportion of cells with +12 on prognosis was assessed by dividing cases into different cutoff values, and we found 60% as the best predictive cut-off value to divide patients with different clinical outcomes. A total of 174 patients (60.2%) presented the +12 in <60% of cells; whereas the remaining 115 patients (39.8 %) carried +12 in ≥60% of cells. Cases with a higher (≥60%) proportion of cells with +12, presented with a higher WBC (P=0.001), lymphocyte count (P=0.006), LDH (P=0.03), size of spleen (P=0.001), and more advanced Binet stage (P=0.04) (Table 1).

Time to first therapy

In our cohort of 289 patients, 175 (60.6%) were treated during follow up, and median TTFT was 42 months (CI95%, 34-49 months). 51.2% of patients in the group with lower proportion of +12 required treatment, compared with 75.7% of patients in the group with ≥60% of cells showing +12 (P<0.001).

As shown in figure 1, we found that median TTFT was 49 months (CI95%, 39-58 months) in cases with +12 in <60% of cells compared with 30 months (CI95%, 22-38 months) in cases with ≥60% (P=0.001). Other significant prognostic variables for TTFT in the univariate analysis were (Figure 1B-H): Binet stage (P<0.0001), B symptoms (P<0.0001), lymphadenopathy (P<0.0001), splenomegaly (P<0.0001), high lymphocyte count (P<0.0001), high B2M (P=0.02), and high LDH (P=0.01) (Table 2). In the multivariate analysis, a shorter TTFT was predicted by Binet stage (P=0.002), ≥60% of cells with +12 (P=0.013), high lymphocyte count (P=0.04), B symptoms (P=0.009) and 11q- in addition to +12 (P=0.002) (Table 3).

Overall survival

At the time of analysis, 69 patients (21.8%) had died. Median OS was 129 months (CI95%, 100-158 months). A total of 16.7% of patients in the group with lower proportion of +12 died compared with 29.6% of patients in the group with ≥ 60% of cells showing +12 (P=0.009) (Table 1).

Regarding OS based on the percentage of cells with +12, cases with less than 60% of cells with +12 had a significantly longer OS,159 months (CI95%, 119-182 months) compared to patients with a higher proportion of cells carrying +12, 96 months (CI95%, 58-134 months) (P=0.015) (Figure 2A). Other variables that also showed a significant impact in OS in the univariate analysis were (Figure 2B-G): advanced Binet stage (P<0.0001), lymphadenopathy (P=0.001), splenomegaly (P=0.001), high LDH

(P=0.009), high (P<0.0001), and CD38 (P=0.04), (Table 4). In the multivariate analysis, only Binet stage (P=0.04), 11q- in addition to +12 (P=0.01) and high B2M (P=0.03) resulted significant in predicting a shorter OS (Table 5).

Sole +12 compared with additional cytogenetic abnormalities

In our group of 289 patients harboring +12, 56 cases (19%) presented additional cytogenetic abnormalities distributed as detailed as follows: 13q- (n=34); 17p- (n=11); 11q- (n=5); 11q- and 13q- (n=3); 17p- and 13q- (n=2); and 17p- and 11q- (n=1); (Supporting information Table S1). Distribution between the different cytogenetic subgroups and the percentage of cells with +12 was similar, as shown in Table 1.

TTFT was shorter in the group of cases with +12 and 11q- compared with cases with +12 as a unique aberration (23 months [CI95%, 9-37] *vs* 44 months [CI95%, 36-52] [P=0.02]) (Supporting information Figure S1A). A shorter OS was observed when simultaneous +12 and 11q- were present (44 months [CI95%, 26-62] *vs* 159 months [CI95%, 92-226] [P=0.02]) (Supporting information Figure S1B).

We could not find any difference in TTFT or OS when +12 was accompanied by 17p- or 13q-. It is remarkable that most of the patients with 11q- (7/9) presented it in ≥ than 25% of 11q deleted nuclei.

Discussion

Cytogenetic abnormalities confer an important prognostic value in CLL [3]. However, recent studies have demonstrated that cytogenetic groups might be heterogeneous, and that the percentage of cells that display a specific abnormality could be related to the prognosis of these subgroups [15, 19, 209]. These observations have not been confirmed before in patients carrying +12. For these reasons, we performed a multicentric analysis of patients diagnosed with CLL and +12 focusing on the prognostic value that the percentage of cells with this abnormality may imply. We observed that patients with +12 constitute a heterogeneous group with intermediate prognosis, with a poor outcome in the subgroup of patients with a higher proportion of cells carrying +12.

Trisomy 12 was present in 13.5% of the patients, being the median OS of 129 months, and median TTFT of 42 months. These findings are consistent with previous publications that estimate frequency of +12 around 15-20%, with a median OS of 111 months, and a median TTFT of 32 months [4]. The other clinical characteristics of the present series, such as the median age (68 years), with a male predominance; the predominance of low lymphocyte count (83%), low $\beta_2$microglobulin levels (71%), early Binet stages (71%), low levels of CD38 (63%), and absence of significant organomegaly, are also in accordance to previous studies [4, 7].

The percentage of cells displaying +12 identified 2 subgroups of patients with different prognosis. Interestingly, patients with a higher proportion of +12 cells presented with higher WBC and lymphocyte count; higher levels of LDH and $\beta_2$microglobulin; more advanced Binet stages; and splenomegaly. More patients with a high proportion of cells with +12 needed treatment or died during follow up.

Of note, the group of patients with +12 in < 60% of their cells, showed a significantly longer TTFT and OS. Moreover, in the multivariate analysis, the independent effect of this covariate remained on TTFT.

To better define prognostic features among all the group of patients with +12, several factors predicting a shorter TTFT were identified. Thus, advanced Binet clinical stage, B symptoms, lymphadenopathy, splenomegaly, high lymphocyte count, and high LDH were associated with shorter TTFT. It is remarkable that the presence of >60% of cells with +12, advanced Binet stage, high lymphocyte count, and B symptoms remained significantly associated with a worse TTFT in the multivariate analysis. These results are similar to the published studies in the overall setting of CLL cases [4, 8, 13].

We also found that similar factors were associated with a shorter OS, including advanced Binet stage, lymphadenopathy, splenomegaly, high LDH, high $\beta_2$microglobulin, and expression of CD38. However, only advance Binet stage, and high $\beta_2$microglobulin remained independently significant in predicting OS.

As speculated with other cytogenetic abnormalities, it may be possible that the greatest number of losses in 13q 11q, or 17p deletions or a high percentage of cells with +12, translates genetic instability that makes the outcome of these patients worse. In our series we found that 60% of cells with +12 was the better cut-off with clinical significance, after trying different thresholds. However, further studies preferably in a prospective context need to be performed to validate this limit.

We could not find predictive value regarding other important prognostic factors such as *IGHV* mutation status, and expression of ZAP-70. Nevertheless, data were collected retrospectively, and these factors were not analyzed in the whole series of patients.

Coexistence of other cytogenetic abnormalities in addition to +12 was rare in our cohort, and it is consistent with previous publications [6, 7, 10]. Only 19% of patients

presented other cytogenetic abnormalities, being 13q- the most frequent followed by 17p- and 11q-. We analyzed TTFT and OS in the different cytogenetic abnormalities, and we found a significantly shorter TTFT and OS in the group of patients with 11q-. This covariate preserved its effect in the multivariate analysis. We failed to observe these findings in the subgroup of patients with 17p-. Moreover, only a tendency to a longer OS in patients with 13q- was observed. It is noteworthy that even though 11q- was only present in 9 patients, nearly all of them presented it in ≥ than 25% of 11q deleted nuclei, which has previously been associated with a worse outcome [8]. However, larger studies with more patients are needed to ascertain these findings, and address the relationship between +12 and other cytogenetic abnormalities.

To summarize, our findings suggest that the percentage of cells carrying +12 influences the outcome of these patients. We demonstrated that a high proportion of cells with +12 detected by FISH is associated with a short OS and TTFT. Our results suggest the need to consider the percentage of cells with +12 as an important prognostic factor, in future prognosis scales.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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Tables

Table 1. Clinical and biological characteristics of the whole series of 289 patients with +12 at diagnosis, according to the percentage of cells with +12 detected by FISH: < 60% or ≥ 60%.

Characteristic	+12 <60% n=174	+12 ≥60% n=115	p value	Global series	%
Age, years. Median (range)	69 (34-88)	68 (25-87)	0.56	68 (25-88)	
Sex					
Male	104	74	0.45	178	61.6
Female	70	41		111	38.4
White blood cells (n=281), (range) x10^9/L	16.2 (4.5-294.2)	23.8 (5.9-276.0)	0.001	19.0 (4.5-294.2)	
Lymphocytes (n=279)					
≤30x10^9/L	149	86	0.006	232	83.2
>30x10^9/L	20	27		47	16.8
LDH (n=276)					
Normal	114	59	0.03	173	62.7
High	54	49		103	37.3
$\beta_2$microglobulin (n=246)					
Normal	110	65	0.08	175	71.1
High	36	35		71	28.9
Binet stage (n=280)					
A	128	71	0.04	199	71.1
B	34	32		66	23.6
C	6	9		15	5.4
Lymphadenopathy (n=244)					
No	70	42	0.34	112	45.9
≤2 areas	31	22		53	21.7
> 2 areas	42	37		79	32.4
Splenomegaly (n=279)					
Yes	15	28	0.001	43	15.5
No	152	84		236	84.5
Hepatomegaly (n=278)					
Yes	7	9	0.38	262	5.7
No	160	102		16	94.3
***IGHV* mutation status (n=80)**					
Mutated	22	19	0.74	37	46.3
Unmutated	24	15		43	53.8
CD38 (n=190)					
Positive	43	28	0.99	71	37.4
Negative	71	48		119	62.6

ZAP-70 (n=95)					
Positive	32	21	0.91	53	55.8
Negative	24	18		42	44.2
Therapy during follow-up (n=287)					
Yes	88	87	<0.001	175	61
No	84	28		112	39
Died during follow-up					
Yes	29	34	0.009	63	21.8
No	145	81		226	78.2
Other FISH abnormalities					
11q- (n= 225)	7	2	0.2	9	3.9
13q- (n=225)	22	17	0.5	39	17
17p- (n=224)	10	4	0.3	14	6.1

Table 2. Univariate Cox regression analysis of time to first therapy (TTFT).

Variable		Median TTFT (months)	95%CI	P value
Percentage of cells with +12	**< 60%**	49	40-58	0.001
	≥ 60%	30	22-38	
Sex	**Male**	33	24-42	0.2
	Female	46	39-53	
Lymphocytes	**≤ 30 x10^9/L**	46	36-56	<0.0001
	>30 x10^9/L	20	15-25	
B symptoms	**Absent**	45	32-55	<0.0001
	Present	7	0-18	
Lactate dehydrogenase	**Normal**	52	36-68	0.001
	High	31	18-43	
$\beta_2$microglobulin	**Normal**	48	36-60	0.002
	High	30	25-35	
Binet stage	**Early**	53	44-63	<0.0001
	Advanced	21	14-28	
Lymphadenopathy	**No**	56	41-71	<0.0001
	≤2 areas	43	25-60	
	> 2 areas	22	16-27	
Splenomegaly	**Absent**	45	37-53	<0.0001
	Present	22	18-27	
Hepatomegaly	**Yes**	40	31-49	0.7
	No	37	24-50	
***IGHV* mutation status**	**Yes**	52	17-87	0.07
	No	31	23-38	

CD38	**Negative**	48	40-56	0.1
	Positive	30	18-42	
ZAP-70	**Negative**	42	16-68	0.4
	Positive	37	23-51	
13q-	**Present**	49	38-59	0.2
	Absent	37	26-47	
11q-	**Present**	23	9-37	0.002
	Absent	44	36-52	
17p-	**Present**	42	20-64	0.3
	Absent	44	35-53	

95% CI, 95% confidence interval.

Table 3. Multivariate Cox regression of time to first therapy (TTFT).

Variable	Hazard ratio	95%CI	P value
+12 ≥60% of cells	1.739	1.126-2.687	0.013
Lymphocytes >30 x10^9/L	1.801	1.025-3.162	0.041
B symptoms	3.038	1.315-7.018	0.009
High $\beta_2$microglobulin	1.426	0.857-2.374	0.17
Advanced Binet stage	2.120	1.311-3.429	0.002
High LDH	1.065	0.684-1.660	0.78
Splenomegaly	0.489	0.201-1.188	0.11
11q-	4.327	1.737-10.782	0.002

95% CI, 95% confidence interval

Table 4. Univariate Cox regression analysis of overall survival (OS)

Variable		Median OS (months)	95%CI	P value
Percentage of cells with +12	**< 60%**	159	100-148	0.015
	≥ 60%	96	58-134	
Sex	**Male**	129	82-176	0.9
	Female	129	Not reached	
Lymphocytes	**≤ 30 x10^9/L**	129	102-156	0.05
	>30 x10^9/L	96	55-137	
B symptoms	**Absent**	129	118-140	0.3
	Present	Not reached	Not reached	
Lactate dehydrogenase	**Normal**	197	Not reached	0.009
	High	81	60-101	
$\beta_2$microglobulin	**Normal**	159	111-207	<0.0001
	High	76	45-107	
Binet stage	**Early**	159	131-187	<0.0001
	Advanced	79	48-109	
Lymphadenopathy	**No**	159	123-195	0.001

	> 2 areas	79	51-106	
Splenomegaly	**Absent**	129	102-155	0.001
	Present	121	Not reached	
Hepatomegaly	**Yes**	129	101-157	0.2
	No	Not reached	Not reached	
IGHV mutation status	**Yes**	159	Not reached	0.6
	No	Not reached	Not reached	
CD38	**Negative**	159	104-214	0.04
	Positive	129	77-181	
13q-	**Present**	129	230-227	0.4
	Absent	159	80-237	
11q-	**Present**	44	26-61	0.018
	Absent	159	92-226	
17p-	**Present**	81	1.1-160	0.4
	Absent	159	91-226	

95% CI, 95% confidence interval

Table 5. Multivariate Cox regression of overall survival (OS).

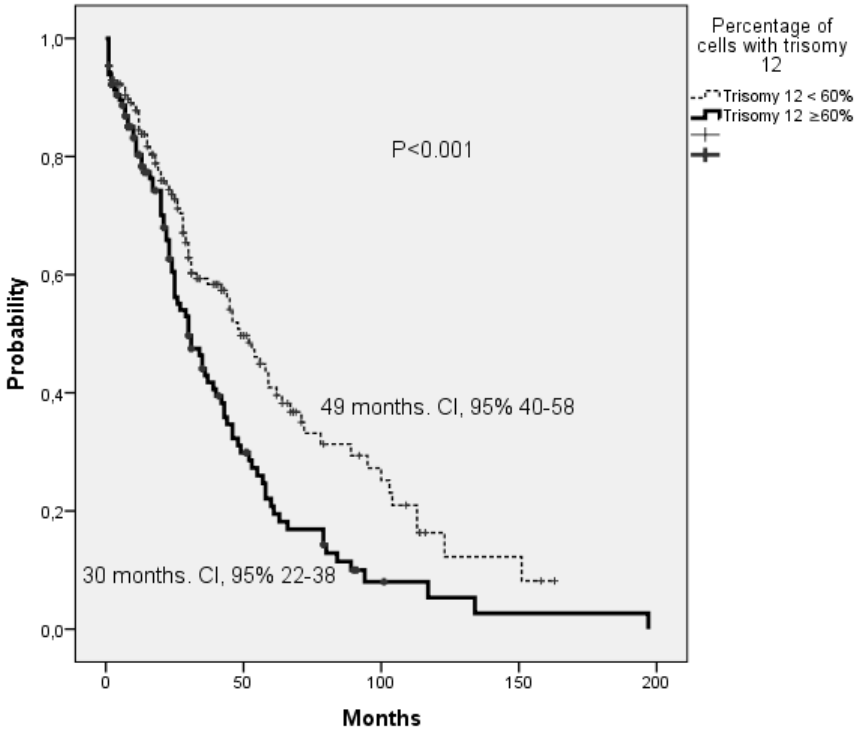
Variable	Hazard ratio	95%CI	P value
+12 ≥60% of cells	1.631	0.834-3.188	0.15
High $\beta_2$microglobulin	2.259	1.070-4.770	0.03
Advanced Binet stage	1.717	1.023-2.881	0.04
High LDH	1.451	0.685-3.074	0.33
11q-	5.097	1.420-18.289	0.01

95% CI, 95% confidence interval

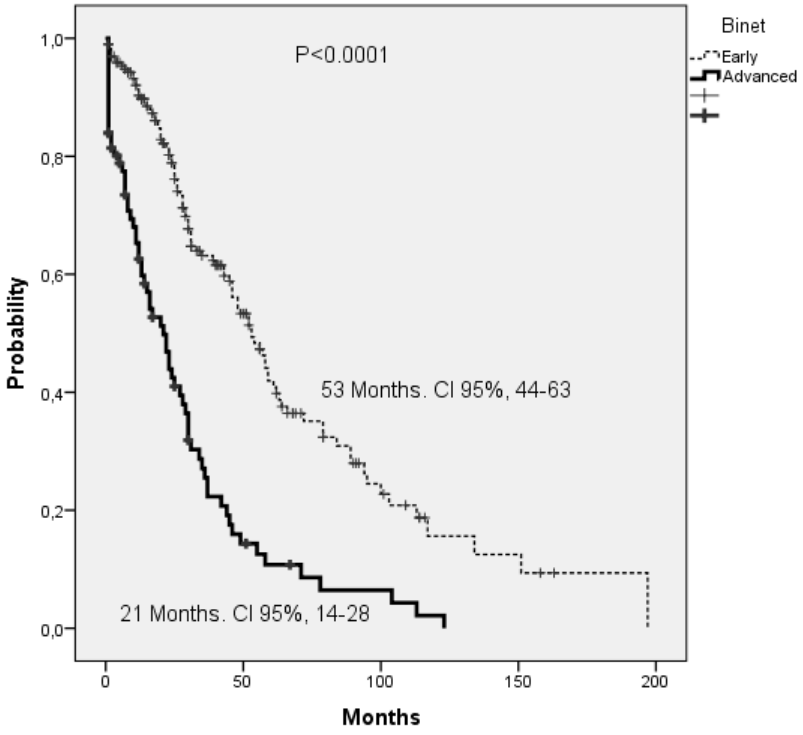
Legends

Figure 1. Kaplan-Meier curves for time to first therapy (TTFT) in 289 patients with +12 and: percentage of +12(< *vs* ≥60%) (A); Binet stage (B); B symptoms (C); Lymphadenopathy (D); Splenomegaly (E); Lymphocyte count (F); LDH (G); and $\beta_2$microglobulin (B2M) (H) (P< 0.005; log-rank test).

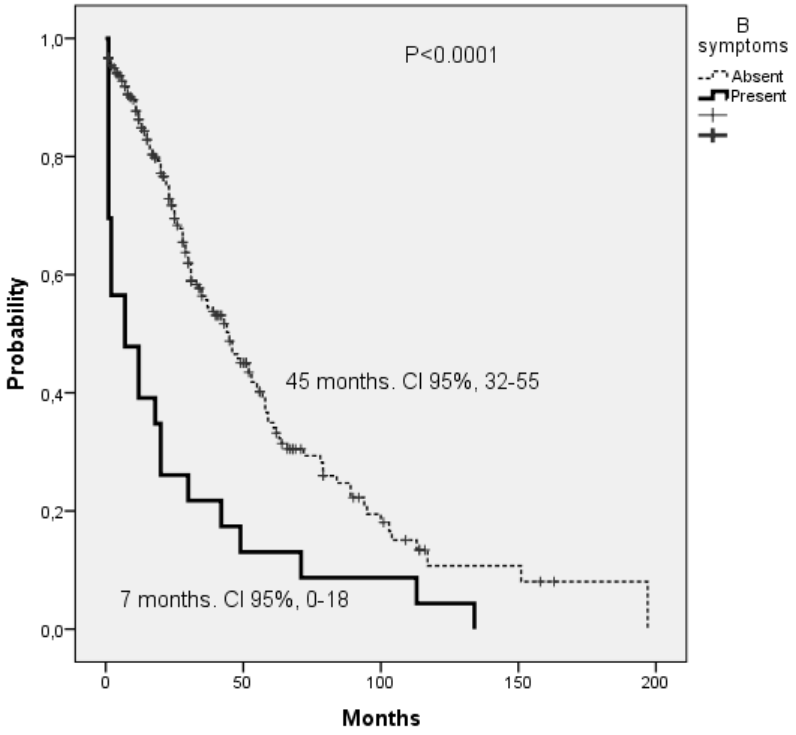
1A


Percentage of cells with trisomy 12
Trisomy 12 < 60%
Trisomy 12 ≥60%
P<0.001
49 months. CI, 95% 40-58
30 months. CI, 95% 22-38
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

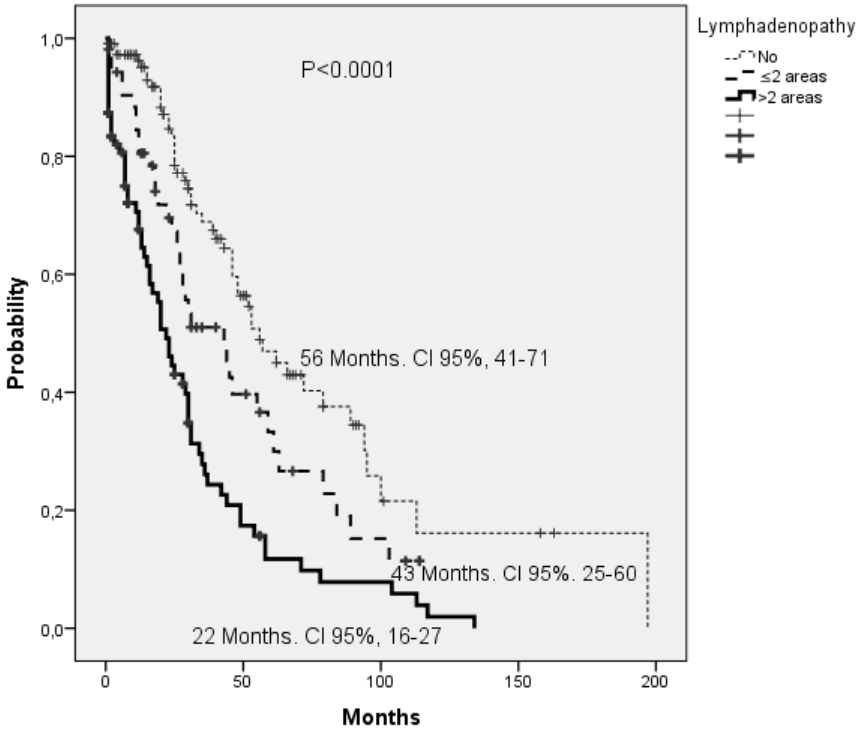
1B


Binet
Early
Advanced
P<0.0001
53 Months. CI 95%, 44-63
21 Months. CI 95%, 14-28
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

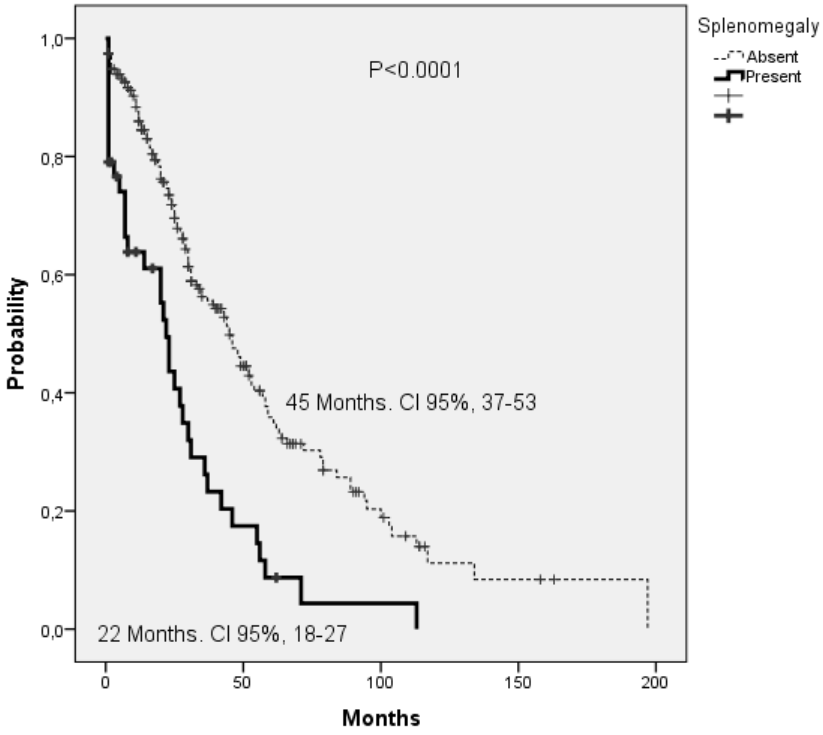
1C


P<0.0001
B symptoms
Absent
Present
45 months. CI 95%, 32-55
7 months. CI 95%, 0-18
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

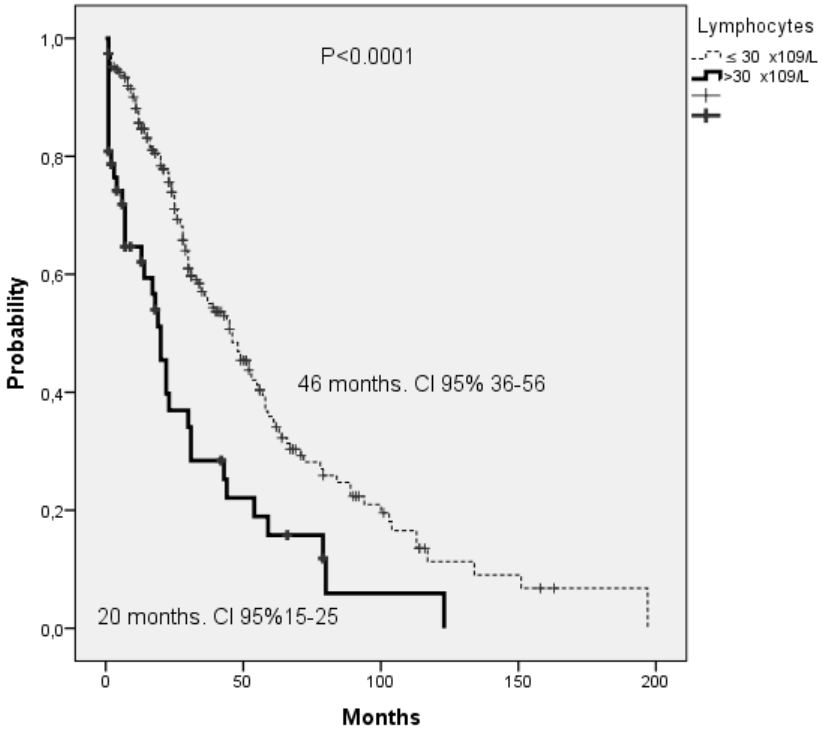
1D


P<0.0001
Lymphadenopathy
No
≤2 areas
>2 areas
56 Months. CI 95%, 41-71
43 Months. CI 95%. 25-60
22 Months. CI 95%, 16-27
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

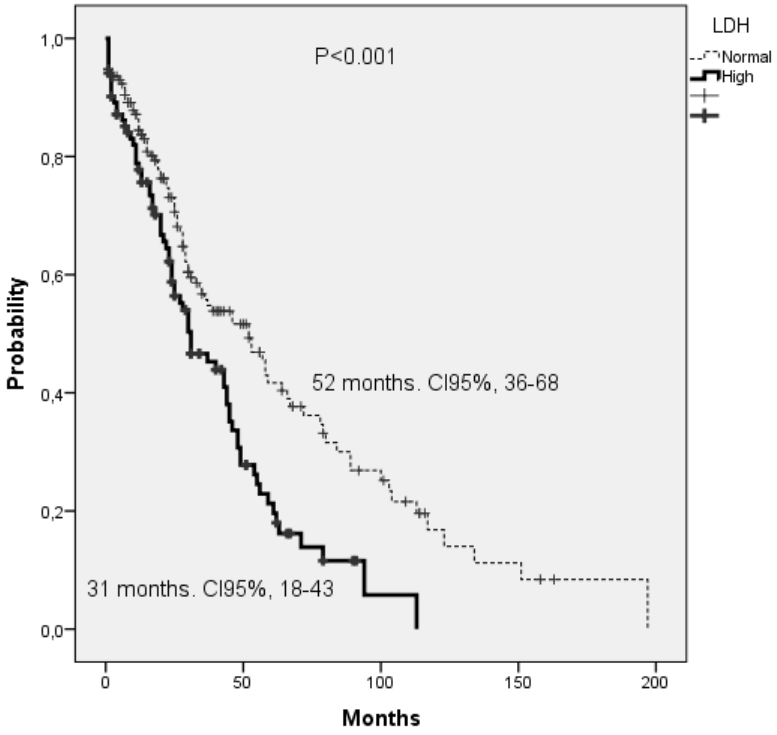
1E


Splenomegaly
Absent
Present
P<0.0001
45 Months. CI 95%, 37-53
22 Months. CI 95%, 18-27
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

1F


Lymphocytes
≤ 30 x109/L
>30 x109/L
P<0.0001
46 months. CI 95% 36-56
20 months. CI 95%15-25
Probability
Months
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200

1G



1H

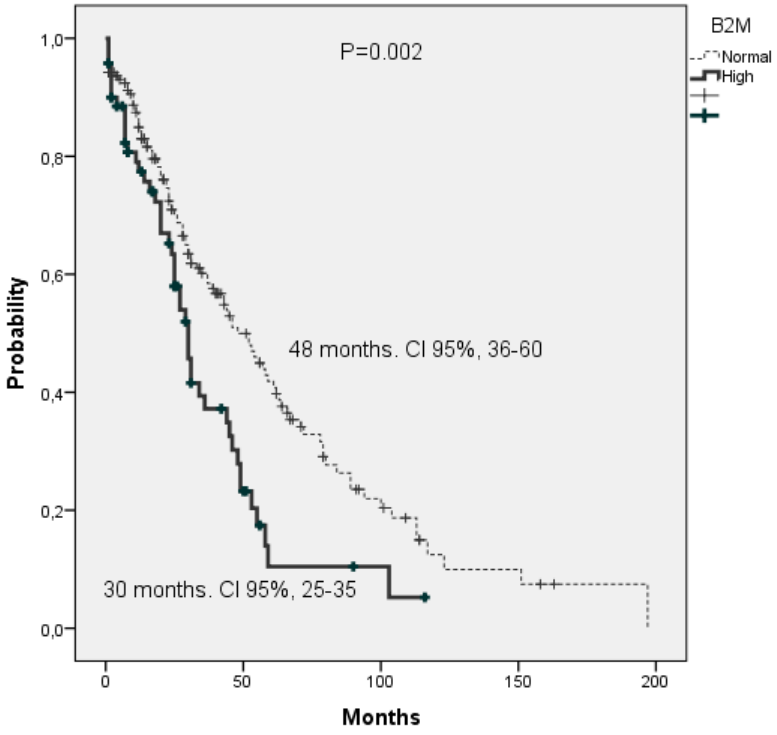
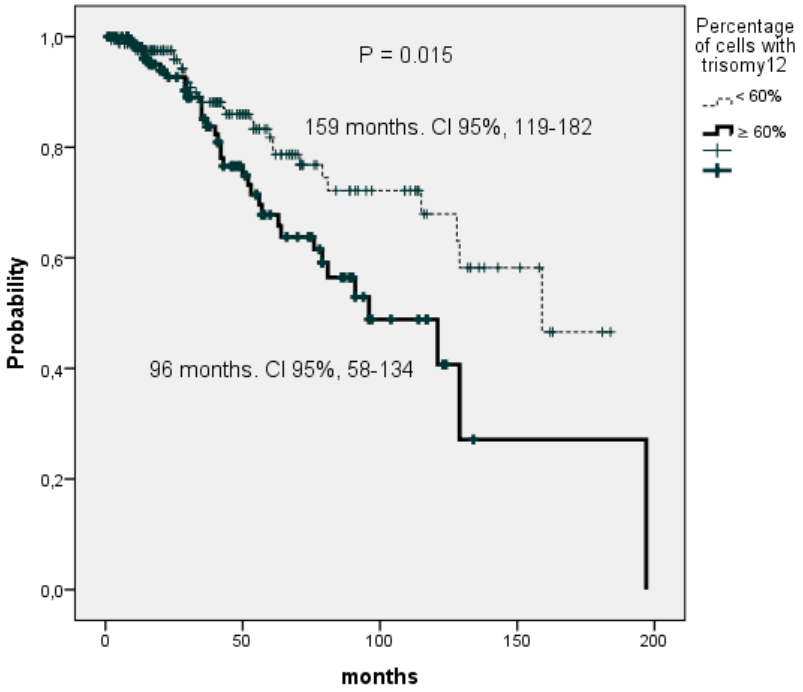
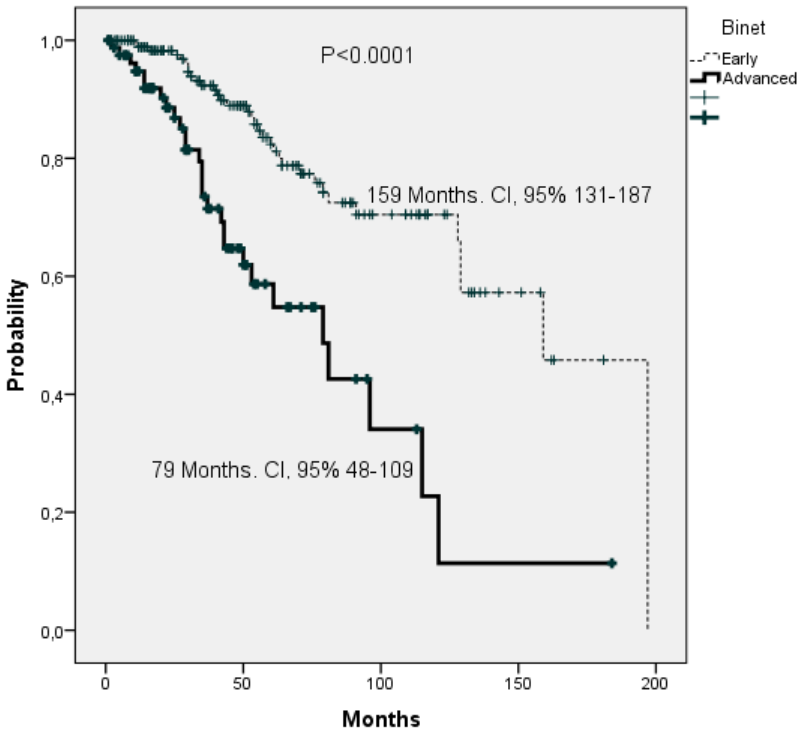


Figure 2 Kaplan-Meier curves for overall survival (OS) in 289 patients with +12 and: percentage of +12 (< *vs* ≥ 60%) (A); Binet stage (B); Lymphadenopathy (C); LDH (D); $\beta_2$microglobulin (B2M) (E); and CD38 (F) (P< 0.005; log-rank test).

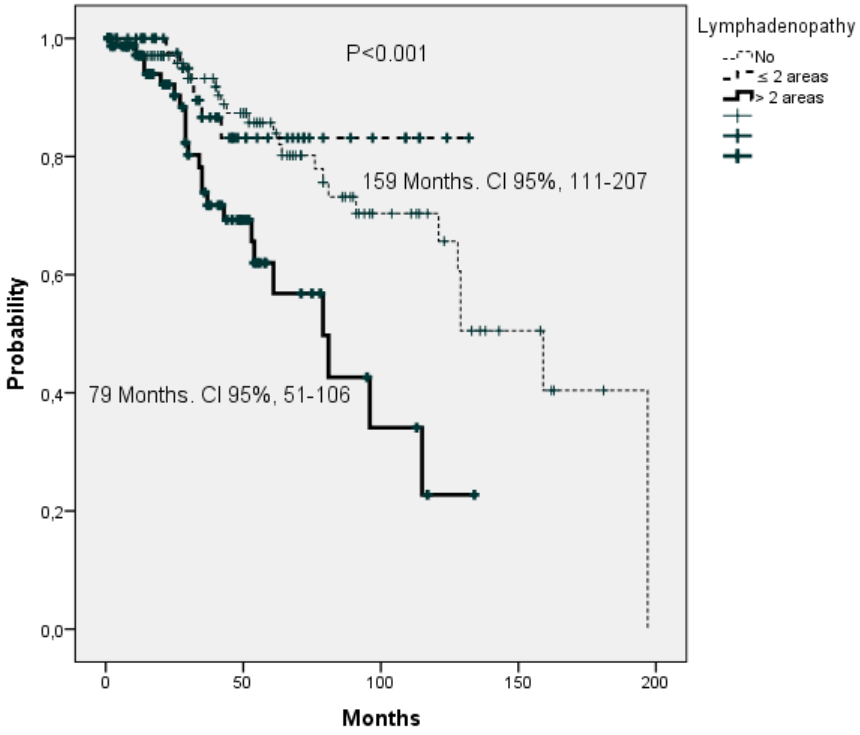
2A


P = 0.015
159 months. CI 95%, 119-182
96 months. CI 95%, 58-134
Probability
months
Percentage of cells with trisomy12
< 60%
≥ 60%

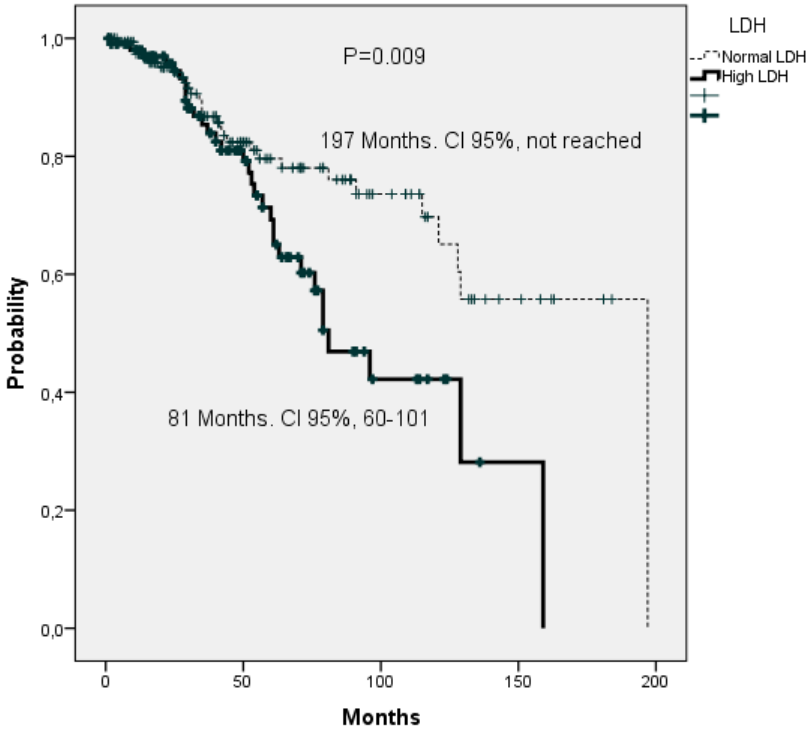
2B


P<0.0001
159 Months. CI, 95% 131-187
79 Months. CI, 95% 48-109
Probability
Months
Binet
Early
Advanced

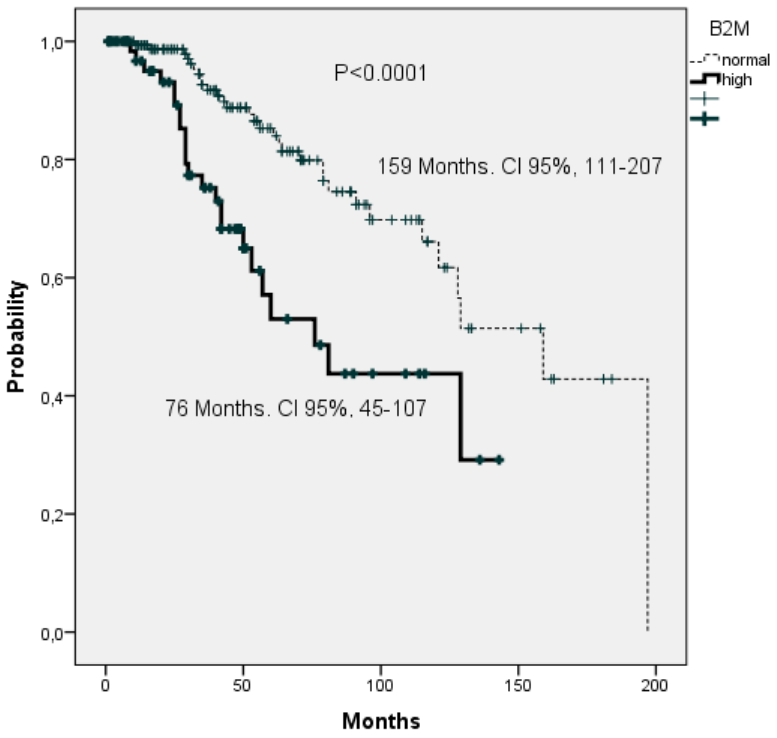
2C


P<0.001
159 Months. CI 95%, 111-207
79 Months. CI 95%, 51-106
Probability
Months
Lymphadenopathy
No
≤ 2 areas
> 2 areas

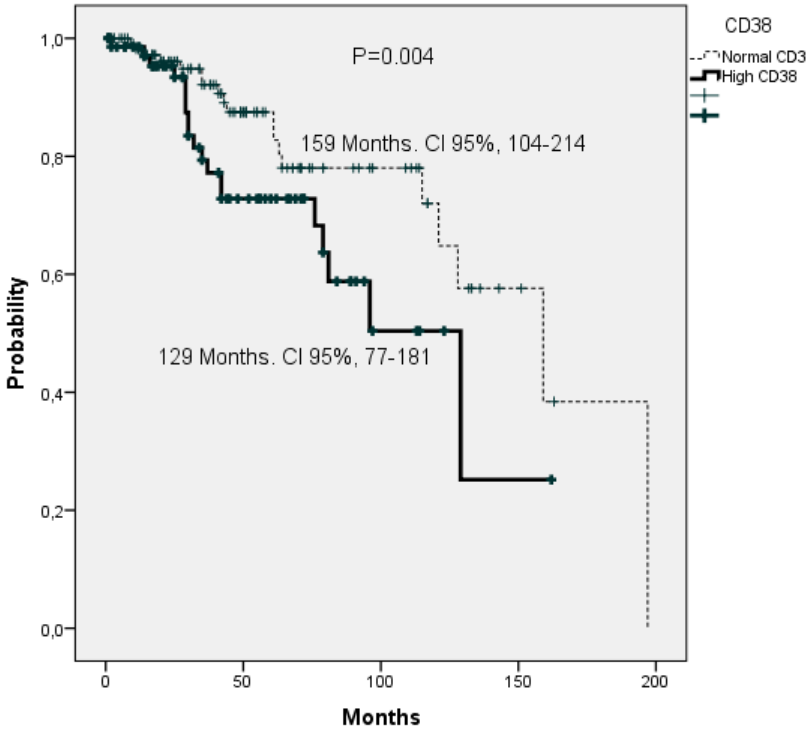
2D


P=0.009
197 Months. CI 95%, not reached
81 Months. CI 95%, 60-101
Probability
Months
LDH
Normal LDH
High LDH

2E



2F



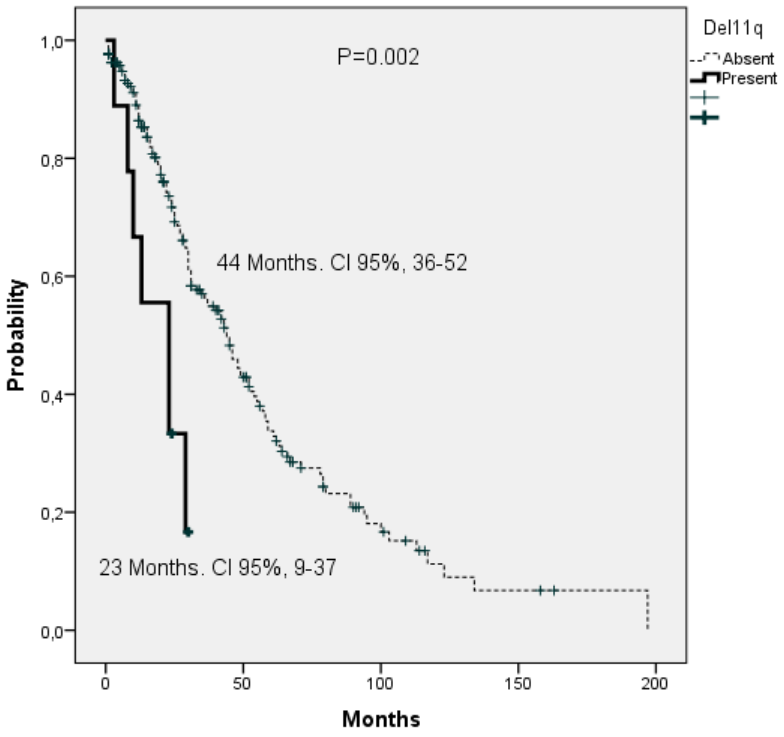
Supporting information

Table S1. Genomic aberrations assesed by FISH divided according to the percentage of cells carrying +12.

	FISH +12 <60% n=174	FISH +12 ≥60% n=115
Total other FISH aberrations	**34**	**22**
13q- (n=34)	17	17
17p- (n=11)	8	3
11q- (n=5)	4	1
11q- and 13q- (n=3)	3	0
17p- and 13q- (n=2)	2	0
17p- and 11q- (n=1)	0	1
Only +12 (n=233)	**140**	**93**

Figure S1. Kaplan-Meier curves for: time to first therapy (TTFT) in patients with +12 and 11q- (A); and overall survival (OS) in patients with +12 and 11q- (B).

S1A



S1B

Supporting information

Table S1. Genomic aberrations assesed by FISH divided according to the percentage of cells carrying +12.

	FISH +12 <60% n=174	FISH +12 ≥60% n=115
Total other FISH aberrations	**34**	**22**
13q- (n=34)	17	17
17p- (n=11)	8	3
11q- (n=5)	4	1
11q- and 13q- (n=3)	3	0
17p- and 13q- (n=2)	2	0
17p- and 11q- (n=1)	0	1
Only +12 (n=233)	**140**	**93**