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The prognostic significance of interphase cytogenetic abnormalities in chronic lymphocytic leukemia

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OBJECTIVE **To assess the frequency of chromosomal aberrations with prognostic significance in a group of 116 previously untreated patients with chronic lymphocytic leukemia (CLL), and to perform risk stratification and compare survival times and time to first treatment (TTFT) between risk groups.** METHOD **Four prognostic groups were formed, based on genetic abnormalities detected by FISH: low risk (del13q), intermediate risk (12q trisomy and no FISH abnormalities), high risk (del11q) and very high risk (del17p). Median overall survival (OS), progression-free survival (PFS) and time to first treatment (TTFT) were assessed for each group using the Kaplan-Meier method and comparisons were made by log-rank test. Within the del13q group survival times and TTFT were compared according to the presence of monoallelic, biallelic deletion or both, and the percentage of cells with the abnormality (≥80% or <80%).** RESULTS **Significant differences were found in the median OS, PFS and TTFT between the four groups, the patients with del17p having the worst prognosis. No significant difference was found in median OS and TTFT between the subgroups of patients with del13q (heterozygous vs homozygous deletions or the percentage of nuclei with the abnormality), but a higher percentage of del13q nuclei was associated with significantly shorter PFS.** CONCLUSIONS **In this analysis, unlike previous studies, patients in the low-risk and intermediate-risk groups had a similar prognosis and may all be considered low-risk. The high-risk patients with del11q may have a better (intermediate) prognosis when treated with immunochemotherapy. These results confirm that patients with CLL bearing del17p should be considered very high risk and are candidates for treatment with novel agents or participation in clinical trials.**

Η προγνωστική σημασία των κυτταρογενετικών ανωμαλιών μεταξύ των διεπαφών σε ασθενείς με χρόνια λεμφογενή λευχαιμία

Περίληψη στο τέλος του άρθρου

Key words

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Risk stratification
Survival

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Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults. It has a highly variable clinical course, with some patients dying within a few years from refractory disease, while others have a normal life expectancy. The clinical Rai and Binet staging systems are useful methods for predicting survival in CLL, but they cannot predict the individual risk of disease progression and survival in the early stages, when the disease is first diagnosed in most patients. The development of interphase fluorescent *in situ* hybridization (FISH) has led to the detection of chromosomal aberrations with prognostic significance in untreated CLL.[1] Based on FISH analysis, five prognostic categories were initially defined:[2] 17p deletion, 11q deletion, 12q trisomy, normal karyotype, and 13q deletion as a sole abnormality. The median survival times reported for patients in these groups were 32, 79, 114, 111, and 133 months, respectively.

In a newer prognostic model, using integrated mutational and cytogenetic analysis, the following risk groups were identified:[3] (a) High-risk, harboring TP53 and or BIRC3 abnormalities (10-year survival: 29%); (b) intermediate-risk – NOTCH1 and or *SF3B1* mutations and or del11q22–q23 (10-year survival: 37%); (c) low-risk – trisomy 12q or normal genetics (10-year survival: 57%); and (d) very low-risk – del13q14 only, whose 10-year survival (69.3%) did not significantly differ from that of a matched general population.

A revised CLL risk stratification model has been suggested in the era of immunochemotherapy, based on both clinical and genetic data.[4] In the "highest-risk" category are

patients with TP53 loss/mutation, who are "purine analog-refractory", with a very short response to prior fludarabine, cyclophosphamide and rituximab (FCR) treatment. These patients are candidates for drugs with proven activity on TP53 deleted/mutant cells, investigational agents in clinical trials or allogeneic SCT. The second "high-risk" group includes fit patients with high β_2-microglobulin, unmutated immunoglobulin heavy chain variable region (IGHV), or 11q deletion. In these patients, the addition of rituximab to FC leads to improved response, and longer progression-free survival (PFS), and overall survival (OS). These patients have a short PFS and may benefit from investigational agents, combined with FCR or maintenance strategies. The "low-risk" patients (no 11q deletion, no TP53 deletion/mutation, mutated IGHV, low β_2-microglobulin, no prior therapy) have a very favourable outcome irrespective of treatment.

According to current treatment guidelines, CLL risk stratification models are clinically relevant only in cases with indications for treatment. "Watch and wait" remains the standard of care for early stage asymptomatic CLL patients irrespective of prognostic factors.

The objective of this study was to assess the frequency of chromosomal aberrations with prognostic significance in a group of patients with CLL, to perform risk stratification based on abnormalities detected by FISH, and to compare survival times and time to first treatment (TTFT) between the stratification groups.

MATERIAL AND METHOD

Retrospective study was made of 116 patients with CLL on whom FISH had been performed, treated in the Department of Hematology between 2006 and 2016. Informed consent for genetic testing was obtained from all subjects, in accordance with the Declaration of Helsinki. The median age of the patients was 62.3 years (range 37–82 years). The characteristics of the patients are summarized in table 1.

Prognostic stratification was performed, based on abnormalities detected by FISH chromosomal. FISH was performed at the time of diagnosis or immediately before starting treatment, using specific probes for the most frequent CLL genetic prognostic markers for CLL: del13q (ON DLEU (13q14)/13qter), 12q trisomy (ON GLI (12q13)/SE12), del11q (ON ATM (11q22)/SE11), del17p (ON p53 (17p13/SE17). Abnormalities detected in 10% or more of the nuclei were considered significant. In 80 cases (68.9%) FISH was performed at diagnosis, and in 25 patients (21.6%) later in the course of the disease at treatment initiation. Of these patients, in 17 (14.7%) cases FISH was performed within 5 years from diagnosis and in 8 (6.9%) more than 5 years from diagnosis. In addition, 11 patients (9.5%) were tested at diagnosis and had a repeated test at treatment initiation. One of them had signs of clonal evolution with detection of del17p.

Table 1. Characteristics of patients with chronic lymphocytic leukemia who underwent FISH (fluorescent *in situ* hybridization) analysis (n=116).

Patient characteristics	Number (n=116)	Percent (%)
Gender		
Male	74	64
Female	42	36
Rai stage		
0	25	22
I	29	25
II	37	32
III	12	10
IV	13	11
Binet stage		
A	68	58
B	24	20
C	25	22
First-line treatment		
None	55	48
Immunochemotherapy*	20	17
Nucleoside-analogue based**	21	18
Alkylating-agent based***	20	17

* Fludarabine, cyclophosphamide, rituximab (FCR) or bendamustine, rituximab (BR)
** Fludarabine, cyclophosphamide (FC)
*** Chlorambucil ± prednisolone; bendamustine monotherapy; cyclophosphamide, vincristine, prednisolone (CVP)

Median survival times were calculated and compared according to the presence of the listed genetic aberrations and the stratification of patients into prognostic groups. Survival times and censored waiting times measured from the date of diagnosis or from the start of first-line treatment (for PFS) were plotted with the use of Kaplan-Meier estimates and curves were compared using the log-rank test. The Pearson Chi-squared test was used to assess the relation between the need for treatment and risk. TTFT was assessed using the Kaplan-Meier method and comparisons were performed by log-rank test. Within the group with del13q, survival times and TTFT were compared according to the presence of monoallelic, biallelic deletion or both, and the percentage of cells with the abnormality (≥80% or <80%). An effect was considered statistically significant if the p value was 0.05 or less. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), v. 22.

RESULTS

Of the 116 patients with CLL, 100 (86%) exhibited FISH abnormalities, which are presented in table 2. In 83 patients

Table 2. Distribution of FISH (fluorescent *in situ* hybridization) abnormalities in 116 untreated patients with chronic lymphocytic leukemia (n=116).

FISH abnormality	Number (n=116)	Percent (%)
Del13q	73	62.9
Monoallelic	41	35.3
Biallelic	5	4.3
Monoallelic+biallelic	11	9.5
With del11q	9	7.8
With trisomy 12q	5	4.3
With del17p	2	1.7
Trisomy 12q	24	20.7
Sole abnormality	18	15.5
With del13q	5	4.3
With del17p	1	0.9
Del11q	12	10.3
Sole abnormality	3	2.5
With del13q	9	7.8
Del17p	8	6.9
Sole abnormality	5	4.3
With del13q	2	1.7
With trisomy 12q	1	0.9
No FISH abnormalities	16	13.8

one aberration was detected and 17 patients had two aberrations. Among the 73 patients with del13q, the deletion was the sole abnormality in 57 (78%). In the remaining 16 patients (22%), del13q was accompanied by 11q deletion, 12q trisomy and 17p deletion. Del11q occurred as a sole aberration in 3 of 12 patients (25%), 12q trisomy in 18 of 23 patients (78%), del17p in 5 of 8 patients (62%). Del13q was monoallelic in 41 of the 57 patients (72%) with del13q as a sole abnormality, in 5 cases (9%) deletions were biallelic, and in 11 patients (19%) there were concomitant monoallelic and biallelic deletions.

The median survival time of the entire group was 12 years, with a 95% confidence interval (CI) of 5–19, and the 5-year survival rate was 84.9%. The estimated 5-year survival rates for the five genetic categories were as follows: 20% for del17p; 68% for del11q; 89% for trisomy 12q; 92% for del13q and 100% for patients with no FISH abnormalities.

The patients were stratified by risk, using the Döhner model,[2] but separating an additional category – the patients with del17p were placed in a "very high-risk" group (fig. 1). The 5-year survival rates for the four groups were as follows: 92% for the low-risk group, 93% for the intermediate-risk group, 68% for the high-risk group and only 20% for the very high-risk group (p<0.001).

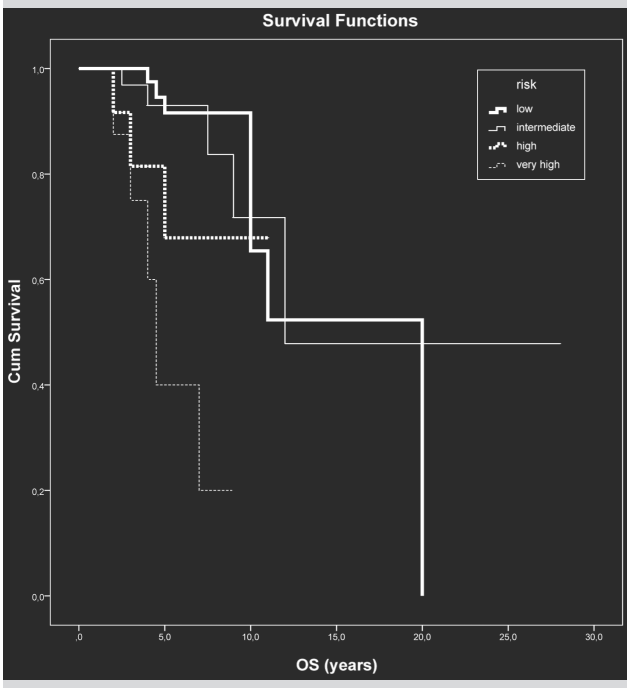

Figure 1. Kaplan-Meier estimates of overall survival (OS) according to risk in patients with chronic lymphocytic leukemia (n=116).

The estimated 5-year disease-specific survival (DSS) rate for the entire cohort was 88.1% (median DSS – 12 years). Only in two patients was death not CLL-related; one in the low-risk and one in the high-risk group. The estimated 5-year DSS rates for the four groups were 91% for the low-risk group, 93% for the intermediate-risk, 74% for the high-risk and 40% for the very high-risk group (p<0.001). The median DSS in the very high-risk group remained unchanged, compared with the median OS (4.5 years).

The very high-risk group had a significantly lower median OS than the low-risk and intermediate-risk groups (log-rank test, p<0.001 and p=0.001, respectively). The differences between the high-risk group and the other three groups (including very high-risk) were not statistically significant (p>0.05).

Different proportions of patients within each prognostic group were indicated for therapy during their disease course. Altogether, 33 (57.9%) of the patients in the low-risk group did not require therapy during the observation period, while in the intermediate-, high- and very high-risk group the numbers were 17 (43.5%), 5 (41.6%) and 0, respectively (Pearson Chi-squared test, p=0.011). Significant differences between the risk groups were also demonstrated in the median TTFT and the 5-year untreated rate (log-rank test, p=0.040), as shown in table 3 and figure 2.

Among treated patients the median PFS was estimated

Table 3. Time to first treatment (TTFT) according to risk in patients with chronic lymphocytic leukemia (n=116).

Risk	Median TTFT (months)	95% confidence interval		5-year untreated rate (%)	p
		Lower bound	Upper bound		
Low	Not reached	.	.	58.4	
Intermediate	48.000	17.853	78.147	40.4	
High	23.000	0.000	58.646	25.0	0.040
Very high	6.000	0.000	16.163	12.5	
Overall	48.000	10.900	85.100	46.6	

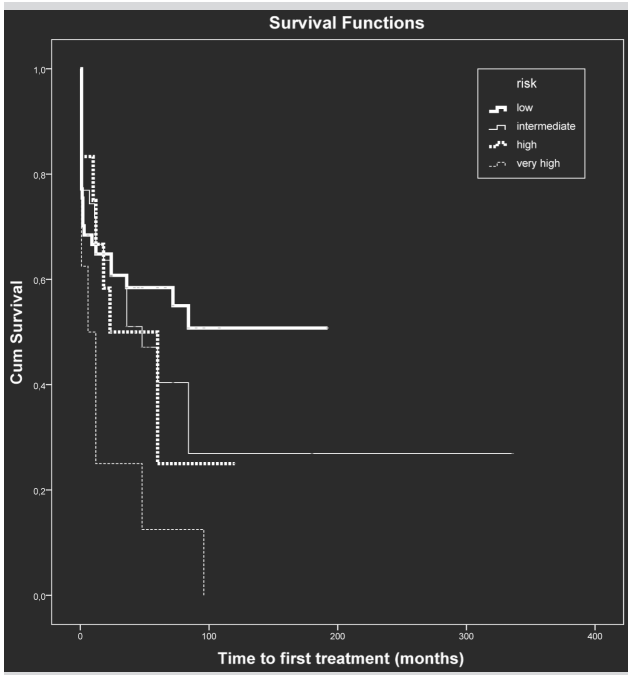

Figure 2. Kaplan-Meier estimates of time to first treatment (TTFT) according to risk in patients with chronic lymphocytic leukemia (n=116).

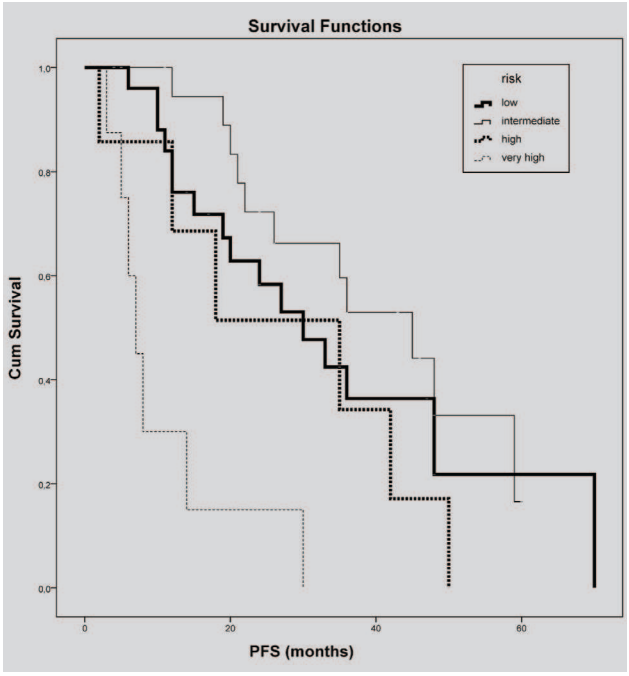

Figure 3. Kaplan-Meier estimates of progression free survival (PFS) in patients with chronic lymphocytic leukemia, according to risk (n=116).

according to the risk, and was 30 months in the low-risk group, 45 months in the intermediate-risk group, 35 months in the high-risk group and 7 months in the very high-risk group (p<0.001, fig. 3).

Patients in the very high-risk group had significantly shorter median PFS than those in the low-, intermediate- and high-risk groups (log-rank test, p<0.001, p<0.001 and p=0.038, respectively).

Additional assessments were made concerning the group of patients with sole del13q (low-risk patients). The 5-year survival rates were estimated in subjects with mono-, biallelic- and combined deletions and no statistically significant differences were found between them (96.4%, 80%, and 85.7%, respectively, p>0.05). TTFT and PFS were also very similar among groups with monoallelic and combined deletions: The 5-year untreated rates were 57.8% vs 47.7% and the 24-month PFS rates were 60.5% vs 60% (p>0.05). The group with biallelic deletions only was rather small (5 patients), of which one patient was indicated for treatment during the observation period.

The percentage of nuclei with del13q was higher in some patients and in order to assess whether this had an impact on survival we set a cut-off value of 80% cells with del13q. The 5-year survival rates of patients with del13q in ≥80% or <80% were similar (80% vs 96.6%, respectively, p>0.05). No significant difference in TTFT was found between the two groups: 5-year untreated rate 45.5% vs 61.6%, respectively, p>0.05 (fig. 4). Among the low-risk patients who ultimately needed treatment, the subjects bearing this abnormality in ≥80% of the cells had significantly shorter PFS (48

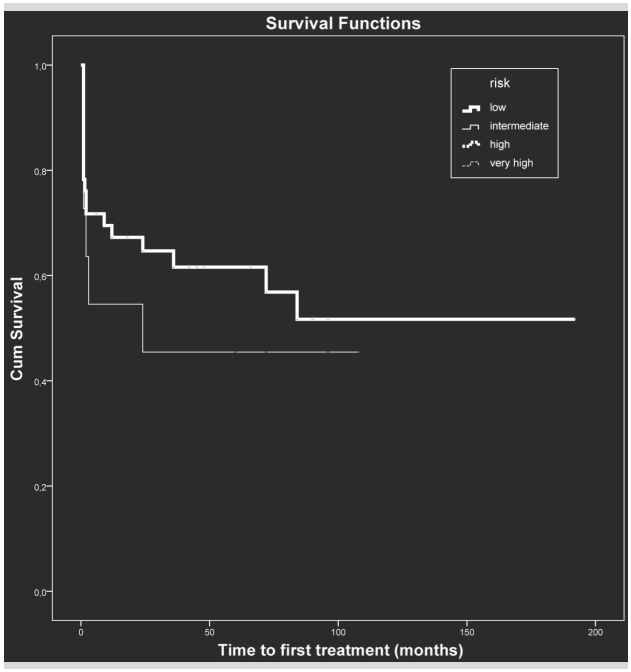

Figure 4. Kaplan-Meier estimates of time to first treatment (TTFT) in patients with chronic lymphocytic leukemia with sole del13q (low risk, n=57) according to the percentage of cells with del13q.

months [95% CI: 25.403–70.597] vs 12 months [95% CI: 0.000–32.404], p=0.033) (fig. 5).

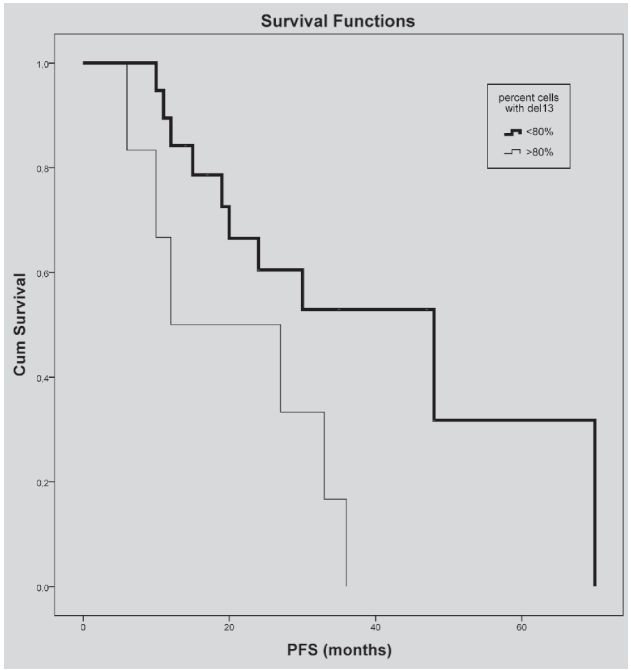

Figure 5. Kaplan-Meier estimates of progression free survival (PFS) in patients with chronic lymphocytic leukemia with sole del13q (low risk, n=57), according to the percentage of cells with del13q.

DISCUSSION

The initial prognostic stratification was based on the Döhner model, according to which patients in the 17p- and 11q-deletion groups have more aggressive disease than those with trisomy 12q, normal karyotype, and 13q deletion as a sole abnormality.[2] Subjects with del17p/TP53 mutations are documented to have the worst prognosis among CLL patients,[3–5] and are often placed in an "ultra-high-risk" or "highest-risk" group. Del17p/TP53 mutations, physical fitness (age), and duration of previous response are the most critical determinants of treatment choice. Based on their efficacy and favorable toxicity profile, novel agents such as ibrutinib, idelalisib, and venetoclax, are the preferred treatment approach for "ultra-high-risk" CLL (del17p/TP53 mutations and refractory/early relapse) patients.[5] Based on these data, del17p and del11q groups were separated and named "very high risk" and "high risk", respectively.

The results confirm that patients with del17p have significantly shorter PFS and TTFT than patients with other FISH abnormalities. With a 5-year survival rate of only 20% these subjects had the worst outcome of all the groups, although the difference between the del17p and del11q patients was not statistically significant (p=0.152). Subjects with del11q had a lower 5-year survival rate than low-risk patients (68% vs 92%), but this difference was not significant. The median PFS of del11q patients was very similar to that of the low/intermediate-risk patients, a result which could be explained by the use of immunochemotherapy. Patients with del13q or trisomy 12q and those with no FISH abnormalities had very similar median OS and PFS and, in the opinion of the authors, should all be considered low-risk.

No differences were found in median OS, PFS and TTFT between patients with mono-, biallelic- or combined del13q. These data are in accordance with the findings of a study in 176 patients with CLL in whom del13q was found, according to which there was no significant difference in survival and TTFT between the groups with mono- and biallelic del13q.[6] A larger analysis in 323 patients[7] also confirms that subjects with heterozygous del13q and those with a homozygous deletion have similar TTFT and OS. In contrast, a higher percentage of del13q nuclei was associated with significantly shorter TTFT (p<0.001). The 5-year untreated rate was 79% for patients with isolated 13q- in ≤65.5% of nuclei compared with 38% among those with 13q- in >65.5% of nuclei (p<0.001). In another trial,[8] the OS was 56 months in the 91 cases in whom ≥80% of cells had loss of 13q (p<0.001). The TTFT in the group with ≥80% vs <80% of loss was 38 months vs 87 months, respectively (p=0.05).

Among the patients in the present study with a sole del13q abnormality, the subjects with ≥80% del13q nuclei had significantly shorter PFS (12 months vs 48 months, p=0.033), but no significant differences in OS and TTFT. The 5-year untreated rate in the group with <80% was higher (61.6% vs 45.5%), though not reaching statistical significance.

Finally, in contrast to previous studies, in the present series the patients with CLL in the low-risk and intermediate-risk groups had a similar prognosis, and may all be considered low-risk. Among patients with del13q as a sole abnormality there was no difference in survival between patients with mono- and biallelic deletions. A higher percentage of del13q (≥80%) nuclei was associated with significantly shorter PFS. The high-risk patients with del11q may have a better (intermediate) prognosis when treated with immunochemotherapy. The study results confirm that patients with CLL bearing del17p should be considered very high-risk. They are not likely to benefit from conventional immunochemotherapy, and therapy with novel agents is indicated or they should be considered as candidate for participation in clinical trials.

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ΠΕΡΙΛΗΨΗ

Η προγνωστική σημασία των κυτταρογενετικών ανωμαλιών μεταξύ των διεπαφών σε ασθενείς με χρόνια λεμφογενή λευχαιμία

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ΣΚΟΠΟΣ Η εκτίμηση της συχνότητας των χρωματοσωμικών ανωμαλιών με προγνωστική σημασία σε μια ομάδα 116 ασθενών με χρόνια λεμφογενή λευχαιμία (ΧΛΛ) χωρίς προηγούμενη θεραπεία και διάκριση του κινδύνου σε σύγκριση με την επιβίωση και τον χρόνο πρώτης θεραπείας μεταξύ των ομάδων (TTFT). **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Με βάση τις γενετικές ανωμαλίες ανιχνευόμενες με FISH δημιουργήθηκαν τέσσερις ομάδες: Χαμηλού κινδύνου (del13q), ενδιάμεσου κινδύνου (τρισωμία 12q και όχι διαταραχές FISH), υψηλού κινδύνου (del11q) και πολύ υψηλού κινδύνου (del17p). Η μέση συνολική επιβίωση (OS), η επιβίωση ελεύθερη προόδου (PFS) και ο χρόνος μέχρι την πρώτη θεραπεία εκτιμήθηκαν για κάθε ομάδα με τη μέθοδο Kaplan-Meier, ενώ οι συγκρίσεις διενεργήθηκαν με τη δοκιμασία log-rank. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Βρέθηκαν σημαντικές διαφορές στη μέση OS, PFS και TTFT μεταξύ των τεσσάρων ομάδων, με χειρότερη πρόγνωση για τους ασθενείς με del17p. Δεν βρέθηκαν στατιστικά σημαντικές διαφορές στη μέση OS και TTFT μεταξύ των υποομάδων των ασθενών με del13q (ετερόζυγη ή ομόζυγη διαγραφή ή το ποσοστό των πυρήνων με την ανωμαλία). Εν τούτοις, υψηλότερο ποσοστό των πυρήνων με del13q συνοδευόταν με σημαντικά βραχύτερο PFS. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Στην ανάλυση της παρούσας μελέτης, σε αντίθεση με προηγούμενες μελέτες, οι ασθενείς των ομάδων χαμηλού ή ενδιάμεσου κινδύνου είχαν παρόμοια πρόγνωση και μπορεί να θεωρηθούν χαμηλού κινδύνου. Οι ασθενείς υψηλού κινδύνου με del11q μπορεί να έχουν καλύτερη (ενδιάμεση) πρόγνωση όταν λαμβάνουν ανοσοχημειοθεραπεία. Τα αποτελέσματά μας επιβεβαιώνουν ότι οι ασθενείς με ΧΛΛ και del17p θα πρέπει να θεωρούνται πολύ υψηλού κινδύνου και ενδείκνυνται για θεραπεία με νεότερα φάρμακα ή για συμμετοχή σε κλινικές μελέτες.

Λέξεις ευρετηρίου: Επιβίωση, FISH, Καθορισμός κινδύνου, ΧΛΛ

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