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# In CLL, comorbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI

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**Short title:**
Comorbidities, complex karyotype and CLL-IPI

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**To the editor**

Chronic lymphocytic leukemia (CLL) represents the most common form of leukemia in Western countries.[1] The clinical course of the disease is quite heterogeneous with some patients living for years with asymptomatic disease and others experiencing early progression and requiring therapeutic interventions.

To allow a rationale management of patients with CLL in clinical practice and in clinical trials, an international prognostic index (CLL-IPI) was defined, based on the relative contribution of the major prognostic parameters, i.e. *TP53* status, *IGHV* mutational status, serum β2-microglobulin, clinical stage, and age.[2]

However, CLL is mainly a disease of the elderly with many patients presenting at diagnosis with significant comorbidities that may affect treatment decisions and outcome.[3] Moreover, in recent years the complex karyotype (CK) emerged as a prognostic biomarker associated with an inferior outcome[4,5] and worse response to treatments including novel drugs.[6,7]

We therefore set out to analyze the prognostic relevance of comorbidities and of the CK in relation to the CLL-IPI.

The study cohort consisted of 335 untreated CLL patients diagnosed and followed at our center between 2006 and 2016 as previously described.[8] All patients were diagnosed and treated according to NCI criteria.[9] The study was approved by the local ethics committee. Fludarabine or bendamustine containing regimens, with or without rituximab were used as first-line treatment in fit patients; chlorambucil with or without rituximab was used in elderly and/or unfit patients according to the treatment policy adopted at our center. Since 2015, ibrutinib or idelalisib plus rituximab were offered to relapsed/refractory patients. Coexisting medical conditions were evaluated according to the cumulative Illness Rating Scale (CIRS) scores as described.[10] Creatinine clearance was assessed with the use of the Cockcroft–Gault formula.[11]

The CK was defined by the presence of at least 3 chromosome aberrations by cytogenetic analysis as described.[12]

The principal clinical and biological characteristics of the patients are reported in supplemental table 1. The median age of these CLL patients was 68.7 years (range 33-96) with 61.5% of the patients older than 65 years. Patients' distribution according to CLL-IPI was as follows: 106 (44.5%) low, 78 (32.8%) intermediate, 43 (18.1%) high and 11 (4.6%) very high. Interestingly, these figures are very similar to those observed for the MAYO cohort of newly diagnosed CLL patients in the original CLL-IPI report[2] and reflect, in our region, a series of patients diagnosed in a center that has a >90% capture of incident CLL cases therefore allowing for meaningful analyses of time to first treatment (TTFT) and overall survival (OS) in a real world scenario. When considering coexisting medical conditions, 145/335 (43.3%) patients had a CIRS score >6. CIRS distribution is reported in supplemental table 2. A creatinine clearance lower than 70 ml/min was present in 136 cases (40.6%). By combining CIRS and creatinine clearance 199/335 (59.4%) of our patients would have been enrolled in the CLL11 trial for CLL patients with coexisting conditions.[13] As expected, a CIRS score >6 was associated with age > 65 years ($p<0.001$), creatinine clearance < 70 ml/min ($p<0.001$), ECOG>=2 ($p<0.001$) and also with β2-microglobulin concentration >3.5 mg/L ($p=0.005$)(supplemental table 3).

A CK was observed in 41/287 (14.3%) of the cases, a figure in keeping with data from recently published series of patients.[5] The CK was significantly associated with advanced Binet stage ($p=0.013$), CD38 positivity ($p=0.003$), β2-microglobulin concentration > 3.5 mg/l ($p=0.010$), TP53 deletion or mutation ($p=0.001$), higher CLL IPI ($p=0.002$) and intermediate unfavorable FISH ($p<0.001$)(supplemental table 4).

In this analysis, we confirmed the prognostic impact of CLL-IPI on OS (table 1, figure 1a) and TTFT (Figure 1d).[14-16] In univariate analysis an inferior OS was also associated with the CK ($p<0.001$;

figure 1b) and a CIRS score >6 (p<0.001; figure 1c). In multivariate analysis, both CK (p=0.002) and CIRS score >6 (p=0.001) confirmed their negative prognostic impact on OS, independently of CLL-IPI. In univariate analysis an inferior TTFT was associated with CK (p<0.001; Figure 1e) but not with CIRS>6. In multivariate analysis the CK retained its negative prognostic impact on TTFT (p=0.012), independently of CLL-IPI. The independent prognostic significance of the CK on TTFT and OS and of comorbidities on OS was also confirmed when CLL-IPI variables were considered separately (supplemental Table 5).

Although larger independent series of patients with longer follow-up are needed to confirm these observations, our findings reinforce the notion that in CLL patients comorbidities and the CK represent novel important prognostic markers. Indeed, relevant comorbidities may shorten life expectancy and may reduce treatment tolerance[3,17] and modern treatment algorithms recommended evaluating not only age, clinical staging and disease-specific prognostic biomarkers, but also comorbidities to guide clinical decisions,[13,18] particularly in the era of novel drugs.[19] However, the prognostic impact of comorbidities and of the CK in the era of mechanism-based treatment needs to be specifically addressed in larger series of patients treated for longer periods of time since in our cohort of CLL these agents were offered only in more recent years.

Although no comorbidity score has been prospectively validated in CLL, the CIRS score is the most frequently used in CLL clinical trials.[13] Furthermore, with the use of effective mitogens, cytogenetic abnormalities and in particular CK recently emerged as one of the novel biomarkers associated with an inferior outcome[4-8,12,20] and with the development of chemorefractoriness.[21]

In conclusion, we showed for the first time that comorbidities and CK were associated with a worse outcome independently of CLL-IPI. We therefore suggest that comorbidities and CK might be considered as additional parameters to be included in CLL prognostic scores for a better management of patients with CLL in clinical practice and in trials evaluating new drugs.

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## Authorship Contributions

Conception and design of the study: GMR, MC, AC.

Data acquisition and patients' follow-up: GMR, MC, FMQ, EL, AU, DF, CL, LF, EG, EV, ET, MAB, AM,

Analysis and interpretation of data: GMR, MC, MN, FC, AC

Writing and review of the manuscript: all the authors contributed to the writing, approval, and review the manuscript.

## Disclosure of Conflicts of Interest

The authors declare that they have no potential conflicts of interests.

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## Tables

### Table 1. Univariate and multivariate analysis for OS and TTFT

| | Univariate analysis | | | Multivariate analysis (n=228) | | | | |
|---|---|---|---|---|---|---|---|---|
| | OS | | | | | | | |
| | | | | | | | After bootstrapping | |
| Variable | HR (95% CI) | p | | HR (95% CI) | p | | HR (95% CI) | p |
| CLLIPI low | 1 | | | 1 | | | 1 | |
| Int | 2.593 (1.121-6.001) | 0.026 | | 2.074 (0.853-5.237) | 0.108 | | 2.074 (0.822-5.049) | 0.122 |
| High | 4.828 (2.152-10.834) | <0.001 | | 5.716 (2.434-13.423) | <0.001 | | 5.716 (2.516-12.989) | <0.001 |
| very high | 13.628 (4.742-39.166) | <0.001 | | 4.875 (1.399-16.984) | 0.013 | | 4.875 (1.161-20.477) | 0.031 |
| CIRS <=6/>6 | 3.843 (2.433-6.071) | <0.001 | | 2.899 (1.521-5.523) | 0.001 | | 2.899 (1.352-6.217) | 0.006 |
| Complex karyotype yes/no | 3.176 (1.882-5.359) | <0.001 | | 3.572 (1.572-8.116) | 0.002 | | 3.572 (1.341-9.515) | 0.011 |
| | | | | | | | | |
| | TTFT | | | | | | | |
| | HR (95% CI) | p | | HR (95% CI) | p | | HR (95% CI) | p |
| CLLIPI low | 1 | | | 1 | | | 1 | |
| Int | 6.640 (2.993-14.729) | <0.001 | | 6.214 (2.788-13.853) | <0.001 | | 6.214 (2.171-17.790) | 0.001 |
| High | 20.831 (9.588-45.260) | <0.001 | | 22.308 (10.214-48.720) | <0.001 | | 22.308 (7.718-64.480) | <0.001 |
| very high | 25.637 (9.748-67.425) | <0.001 | | 15.811 (5.611-44.555) | <0.001 | | 15.811 (4.425-56.502) | <0.001 |
| CIRS <=6/>6 | 1.151 (0.794-1.669) | 0.407 | | - | - | | - | - |
| Complex karyotype yes/no | 2.521 (1.606-3.958) | <0.001 | | 2.157 (1.185-3.926) | 0.012 | | 2.157 (1.177-3.952) | 0.013 |

Note

Time to first treatment (TTFT) was calculated as the interval between diagnosis and the start of first line treatment.

Overall survival (OS) was calculated from the date of diagnosis until death due to any cause or until the last patient follow-up.

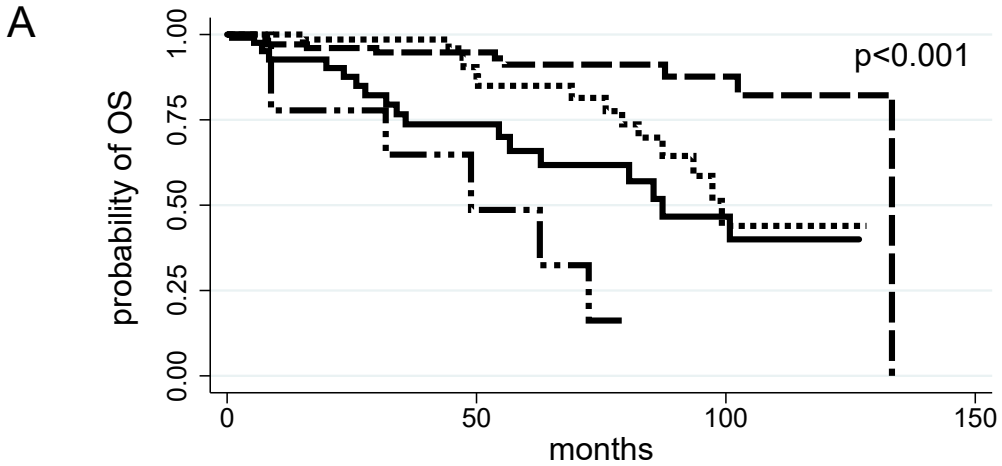
Proportional hazards regression analysis was used to identify the significant independent prognostic variables on TTFT.

The stability of the Cox model was internally validated using bootstrapping procedures.[4]

Statistical analysis was performed using Stata 14.0 (Stata Corp, College Station, TX).

**Figure Legends**

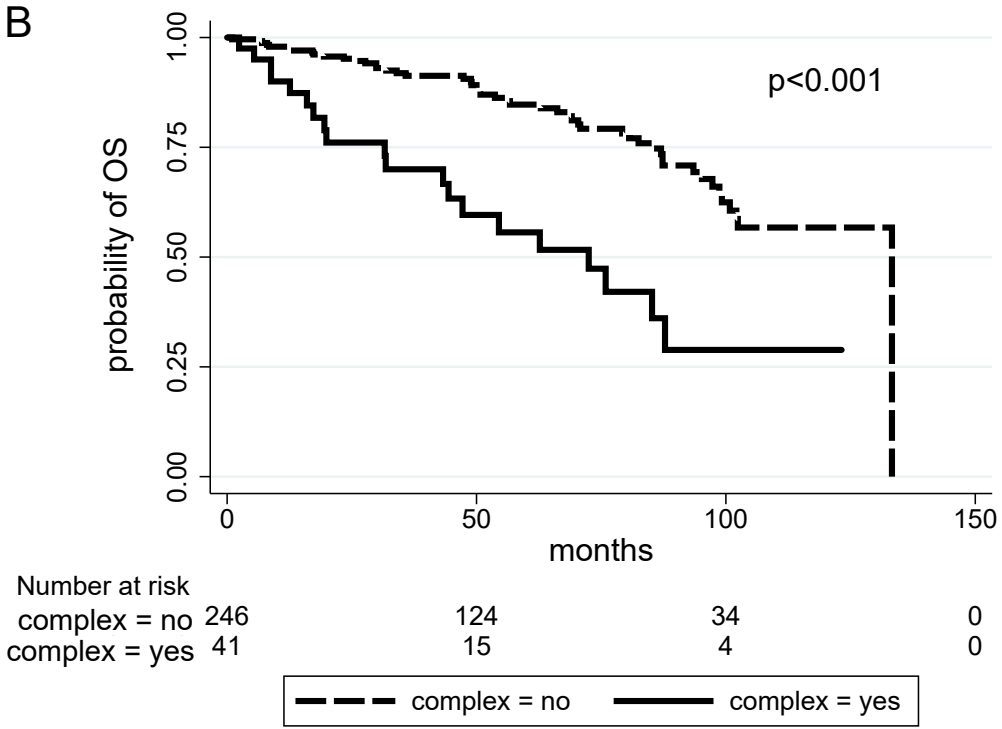
Figure 1.
OS according to CLL-IPI (a), complex karyotype (b) and CIRS (c). TTFT according to CLL-IPI (d) and complex karyotype (e).

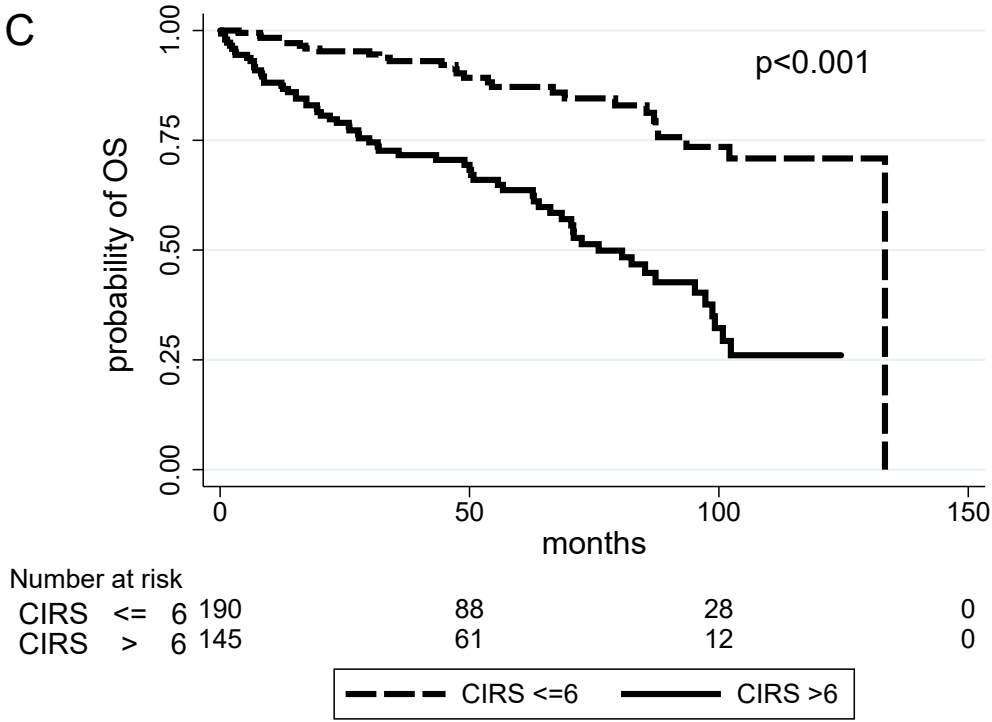


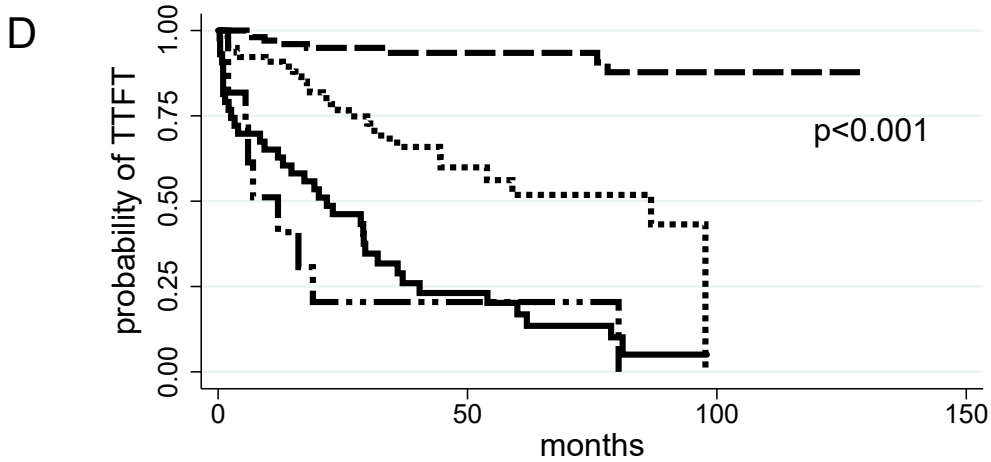

Number at risk

| | | | | |
|---|---|---|---|---|
| CLL-IPI low | 106 | 55 | 17 | 0 |
| CLL-IPI int | 78 | 33 | 6 | 0 |
| CLL-IPI high | 43 | 20 | 7 | 0 |
| CLL-IPI very high | 11 | 3 | 0 | 0 |

CLL-IPI low
CLL-IPI int
CLL-IPI high
CLL-IPI very high


B
probability of OS
1.00
0.75
0.50
0.25
0.00
p<0.001
0
50
100
150
months
Number at risk
complex = no 246 124 34 0
complex = yes 41 15 4 0
complex = no
complex = yes

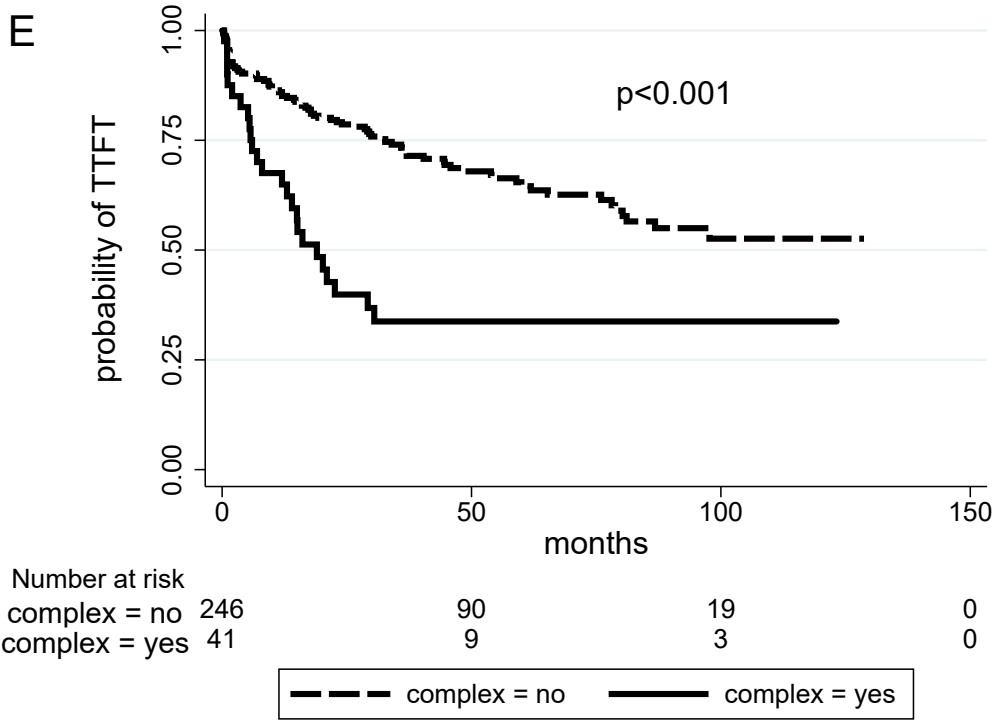

C
p<0.001
probability of OS
0.00
0.25
0.50
0.75
1.00
0
50
100
150
months
Number at risk
CIRS <= 6 190 88 28 0
CIRS > 6 145 61 12 0
CIRS <=6
CIRS >6




Number at risk

| | | | | |
|---|---|---|---|---|
| CLL-IPI low | 106 | 53 | 16 | 0 |
| CLL-IPI int | 78 | 18 | 0 | 0 |
| CLL-IPI high | 43 | 8 | 0 | 0 |
| CLL-IPI very high | 11 | 2 | 0 | 0 |

CLL-IPI low
CLL-IPI int
CLL-IPI high
CLL-IPI very high


E
probability of TTFT
1.00
0.75
0.50
0.25
0.00
p<0.001
0
50
100
150
months
Number at risk
complex = no 246 90 19 0
complex = yes 41 9 3 0
complex = no
complex = yes

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