

REVIEW

COMPLEX KARYOTYPE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Received: 13 June 2023

Revised: 30 June 2023

Accepted: 07 July 2023



Check for
updates

Funding information:

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Competing interests:

The authors have declared that no competing interests exist

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Summary

Chronic lymphocytic leukemia (CLL) is a genetically heterogeneous disease with chromosomal and genomic aberrations found in more than 80% of patients, either by conventional or by molecular cytogenetics. Complex karyotype (CK) is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of CLL malignant cell and is considered a potential prognostic parameter in CLL. The detection of CK in CLL patients can potentially affect prognosis and treatment, considering that CK is associated with the progression of HLL and a worse prognosis, as well as with a higher risk of developing Richter transformation. This review will assess the complexity of karyotype analysis in CLL and its prognostic importance and implications.

Key words: chronic lymphocytic leukemia, complex karyotype, chromosomal aberrations

Cite this article as: Antić D, Kozarac S, Tomić Vujović K, Otašević V, Pešić A, Vuković V, Mihaljević B. Complex karyotype in chronic lymphocytic leukemia. Medicinska istraživanja 2023; 56(3):77-85 10.5937/medi56-45003

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disease of typically CD5 positive B cells in the blood, bone marrow, lymph nodes, or spleen, and it represents the most common form of leukemia that affects the elderly population (1–3). CLL is a genetically heterogeneous disease with chromosomal and genomic aberrations found in more than 80% of patients, either by conventional or by molecular cytogenetics (1,4). Most common aberrations found in CLL patients are deletion in chromosome 13q (del(13q)), deletion in chromosome 11q (del(11q)), deletion in chromosome 17p (del(17p)), or trisomy 12 (1–9). Deletion of 6q, 2p gain, 8q gain, deletion of 14q, deletion of 15q, trisomy 18, trisomy 19, and others are found, but less frequently (6).

Genetic diversity of CLL reflects clinical heterogeneity, with significant variation in clinical course among patients. To enhance our understanding of CLL and develop effective prognostic models, multiple prognostic factors have been identified and studied (10). These factors include various genetic and clinical parameters that aid clinicians in predicting a patient’s clinical course and risk profile. As it was shown during the COVID-19 pandemic, due to compromised immune function and increased susceptibility to severe infectious complications, CLL patients may be particularly vulnerable, which is why, it is an imperative to identify CLL patients who exhibit poor prognostic factors (11).

TP53 mutation (del(17p)) and mutational status of immunoglobulin heavy variable gene (IGHV) are widely recognized as standardized prognostic factors in CLL, with significant clinical implications for predicting disease progression and overall survival (1, 6, 10). These factors have been incorporated into clinical and research guidelines for the management of CLL patients and are particularly relevant in determining the most appropriate treatment strategy, such as choosing between chemoimmunotherapy and targeted therapy.

While previously mentioned established prognostic factors have been proven to be clinically valuable, they are not without limitations. New and more accurate prognostic factors may be needed to capture the heterogeneity of CLL, provide more accurate predictions, and guide personalized treatment decisions (12-13). Advances in genomics and other technologies have made it possible to identify new prognostic factors, such as complex karyotype (CK), tumor microenvironment, epigenetic changes, and others (12-15).

Complex karyotype in CLL is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of a CLL malignant cell (4,5). Since CK occurs in approximately 20% of untreated CLL patients, the question of its prognostic value is becoming more important (5). This review will assess the complexity of karyotype analysis in CLL and its prognostic importance and implications.

DETECTION OF CHROMOSOMAL ABERRATIONS

The fluorescence in situ hybridization (FISH) analysis has become a standard diagnostic procedure in patients with CLL, carried out to detect the four most common aberrations, including the del(17p), one of the main prognostic parameters in CLL. Still, FISH, which is mainly used for the detection of chromosomal aberrations, cannot provide a complete overview of the cytogenetic landscape of CLL (2, 3, 16).

Conventional cytogenetic methods, such as chromosome banding analysis (CBA), offer an assessment of a malignant CLL clone. CBA provides single-cell analysis, detection of balanced chromosomal rearrangement, and detection of clonal evolution. Understanding clone characteristics can potentially affect prognosis and treatment (3, 16). However, CBA was not introduced in routine practice with respect to CLL, unlike in the case of acute leukemias and myelodysplastic syndromes, primarily due to insufficient in vitro proliferative capacity of CLL cells, resulting in poor sensitivity of this method with regard to the detection of abnormal clones (16). After the issue of CLL culture growth had been successfully overcome, the use of conventional cytogenetics expanded and potentiated better detection of aberrations.

Although FISH has overcome the limitations of CBA, these methods complement each other, since there are prognostically significant aberrations that cannot be identified using a single technique. The use of chromosome microarray analysis (CMA) in CLL also provides the whole genome scan but cannot identify balanced chromosome rearrangements (17). Recent recommendations by Jondreville et al. for karyotype and FISH analysis in CLL are shown in **Table 1**.

Recent CLL guidelines suggest FISH, TP53 gene analysis, and IGHV mutational status in general practice.

Table 1. Recent recommendations for karyotype and FISH analysis in CLL

	Clinical practice	Clinical trials
On diagnosis		
Karyotype	recommended	mandatory
FISH – 4-probe ¹	recommended	mandatory
FISH – other probes	depending on karyotype ²	depending on the purpose ³
Before treatment		
Karyotype	mandatory	mandatory
FISH – 4-probe ¹	mandatory	mandatory
FISH – other probes	depending on karyotype ²	depending on the purpose ³

¹ Detection of del(13)(q14)(D13S319), +12, del(11)(q22)(ATM) and del(17)(p13)(TP53)

² Detection/confirmation of other chromosomal abnormalities (within CK or not) with a prognostic impact (e.g., 2p gain, 8q gain, 8p deletion)

³ Other probes depending on the chromosomal abnormality of interest in clinical trial

CBA analysis is recommended only in the clinical trials setting, since the significance of the CK is still under investigation.

A CLOSER LOOK AT CONVENTIONAL CYTOGENETIC METHODS

In the past, most CLL cases had a very low mitotic index, even in the presence of B-cell mitogens (polyclonal B-cell activators including Epstein-Barr Virus (EBV), lipopolysaccharide from *E. coli* (LPS), pokeweed, CD-40 ligand, and/or different interleukins). Therefore, the use of metaphase (conventional) cytogenetics was very limited. However, those results led to the discovery of recurrent cytogenetic aberrations in CLL. Those findings were implemented in a much simpler method for analyzing genetic aberrations in CLL – interphase FISH.

A significant improvement in conventional cytogenetics in CLL was the introduction of immunostimulatory CpG oligonucleotide DSP30 in combination with interleukin -2 (IL-2). This combination induces cell cycle progression of CLL cells *in vitro* and provides sufficient mitoses for conventional cytogenetics in more than 80% of CLL patients (18). Dicker et al. used 10^7 peripheral blood mononuclear cells that were cultured in 5 mL RPMI 1640 medium (Gibco) with 20% fetal calf serum, DSP30 (2 μ M) (TIB MolBiol) and IL-2 (200U/ml) for metaphase induction. After 48 hours, colcemid (Sigma) at a concentration level of 0.15 g/mL was added for another 24 hours before chromosome preparation. Chromosome preparation and staining was done according to standard protocols. Chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN). In three cases, peripheral blood and bone marrow were available from the same patients, which showed that cell culture with DSP30/IL-2 resulted in the detection of the same aberrations on metaphases from different sources. Therefore, peripheral blood was shown to be an adequate sample for conventional cytogenetic analysis, with the procedure being more comfortable for patients.

Baliakas et al. tested protocols used for metaphase induction based on either phorbol-12-myristate-13-acetate (TPA) or immunostimulatory CpG-oligonucleotide DSP30 plus IL-2 following standard procedure and concluded that no difference regarding the number of obtained metaphases was observed between the two protocols (3).

According to the latest recommendation of the ERIC (European Research Initiative on CLL) from 2022, the most appropriate source of tumor cells for conventional cytogenetics is peripheral blood on heparin, as it usually has a high CLL cell fraction. A total of 2×10^6 leukocytes/mL medium are cultured in medium with 20% fetal calf serum and mitogens. It is recommended that 2 parallel cultures with different cell mitogens be set up for each

patient, one with 12-O-tetradecanoly-phorbol-13-acetate (TPO), and the other with IL-2 plus DSP30. CLL cells remain in culture for 72 hours, after which anti-mitotic colcemid is added to the media to obtain metaphases. Upon incubation, harvesting of the cultures is performed following standard cytogenetic procedures: hypotonic solution and fixation with Carnoy's solution (3: 1 = methanol:acetic acid). Finally, a cell suspension is obtained, adjusted to an optimal cell concentration, and slides are prepared. After that, banding and staining is carried out using trypsin and Giemsa. Metaphases should be screened with a microscope or captured using a metaphase finder. A minimum of 20 metaphases should be analyzed in cases with a normal karyotype. Ten metaphases should be fully analyzed, with additional 10 analyzed or counted and scored, for relevant structural chromosomal aberrations (16).

THE COMPLEX KARYOTYPE – DEFINITION

The CK in CLL is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of a CLL malignant cell, found in 2 out of 20 cells. The presence of ≥ 5 abnormalities is considered to be high CK (3,12). Nonetheless, cytogenetic analysis interpretation can be challenging; therefore, it is recommended that cytogeneticists count aberrations in order to enable clinicians to draw a clear conclusion. Guidelines for counting aberrations in karyotype suggest counting every aberration in every clone and subclone. A single change should be counted only once if it is present in more than one clone. Additionally, special interest should be devoted to distinguishing between the CK with 3, 4, and ≥ 5 aberrations (6).

Based on the existing data, it is evident that CLL heterogeneity also exists within the CK group, and that not all CKs have the same level of significance (3, 5, 9). The number and type of abnormalities, as well as the effects of clonal selection resulting from the treatment, are some of the factors that seem to influence the clinical relevance of CK in CLL. Therefore, it is important to consider a specific CK profile of each individual patient rather than just the number of abnormalities when assessing the patients' prognosis and making treatment decisions.

The number of chromosomal aberrations in CK signals different prognoses in CLL. More specifically, patients with ≥ 5 abnormalities (high-CK) have a very poor outcome, with a median overall survival of 3.1 years. This is independent of clinical stage, TP53 aberrations, and IGHV gene somatic hypermutation status (3, 9). Patients with 3 or 4 aberrations (low-CK and intermediate-CK, respectively) have a shorter survival (median OS of 4.3 years) only when accompanied by TP53 aberrations (3, 9). Furthermore, there are patients with ultra complex karyotypes, having ≥ 10 or ≥ 15 abnormalities, who have particularly poor survival (20).

Besides the number, a type of chromosomal aberrations in CK also affects its prognostic features. An example of this is patients with +12 and +19 aberrations that fulfil criteria for CK but are characterized by an extremely indolent course with prolonged time to the first treatment (TTFT) and OS which is longer than any of the other CK cases or cases without CK, including other M-CLL (3, 9, 16). Interestingly, they have peculiar clinical features (i.e., female predominance, young age at diagnosis, etc.) and comprise nearly 10% of all CK ≥ 3 cases. CK may also reflect clonal complexity, i.e., the presence of subclones. In one study, co-occurrence of CK and clonal aberrations was found in 74% cases, which significantly affects the outcome in CLL patients (22, 23). The effect of various disease characteristics and treatment options on the impact of CK in CLL are shown in **Table 2**. Disease features as well as different treatment options may either aggravate the negative impact of complex karyotype (listed in the second column), or have a neutral effect (listed in the third column). (26)

CK may occur at the time of diagnosis, in relapse, or in the progression of the disease. CK has been observed in up to 20% treatment-naive patients and in up to 40% patients with R/R CLL (5, 20, 24). There is a scarcity of research on sequential karyotype analysis in patients with CLL, but one study has found that the analysis can reveal clonal evolution by means of chromosome analysis in nearly half the patients (45.8%) who remain untreated for 24 months (20, 24). Moreover, patients who exhibit clonal evolution are at a higher risk of disease progression, which underscores the importance of monitoring chromosomal changes over time in CLL patients.

The exact cause of CK in CLL is uncertain. In patients with TP53 mutation/deletion, genome instability leads to clone evolution and detection of CK in progression or in relapse (4). In U-CLL, it has been suggested that the origin of CK development lies in an enhanced lymphocyte response to antigens, which leads to the stimulation of intracellular B-cell-receptor (BCR) signaling and proliferation. Next, during each cell division, telomeres shorten, promoting the development of genetic lesions. Genes implicated in the DNA repair (e.g., TP53, ATM), in ubiquitin-mediated degradation of oncoproteins (e.g., FBXW7), and in the inflammatory pathway (e.g.,

MYD88) could be affected as well, which further increases the risk of chromosome breaks (26).

PROGNOSTIC VALUE OF THE CK IN CLL

Identification of complex karyotype in CLL patients may assist in risk stratification, based on the results of studies that have evaluated the prognostic value of CK, including its association with other poor outcome prognostic factors, as well as its value as a single factor of adverse prognosis. These studies have shown that unfavorable prognostic factors, such as unmutated IGHV status, del(17p), TP53, and del(11q), are commonly seen in CLL patients with CK (3, 5, 6, 17, 27-29). Apparently, the interaction between CK, TP53 mutation, and IGHV mutational status is very complex.

An association between complex karyotype and unmutated IGHV status in CLL patients has been demonstrated in some studies, with the latter being indicative of poorer prognosis relative to mutated IGHV status (3, 5, 6, 17, 27, 29, 30). A comprehensive study by Baliakas et al. involving 5290 CLL patients found that 72% of patients with complex karyotype (CK) had unmutated IGHV status, while only 28% of patients with a normal karyotype had unmutated IGHV status (3). Biological mechanisms underlying this relationship, however, remain incompletely understood. It is hypothesized that genetic alterations leading to a complex karyotype may disrupt the signaling pathways involved in IGHV gene expression and somatic hypermutation, thereby contributing to the development of an unmutated IGHV status. On the other hand, in M-IGHV patients without any CK subtypes at diagnosis the disease is characteristically very indolent, with a median TTFT of 19 years, and with more than 90% of patients being alive 10 years upon the diagnosis (3, 29).

The presence of a complex karyotype in CLL patients is strongly associated with TP53 aberrations, with up to 80% of patients exhibiting both abnormalities (3, 5, 17, 27-29). This co-occurrence is of significant clinical concern, as it is associated with a very poor prognosis and limited treatment options. It has been observed that CK CLL patients with TP53 abnormalities have unsatisfactory

Table 2. The clinical significance of complex karyotype in CLL

Effect on impact of CK	Negative	Neutral
Disease features / type of treatment	High CK	CK with +12, +19
	TP53 aberrations in low - CK and intermediate - CK	low - CK and intermediate - CK without TP53 aberrations
	U - CLL	M - CLL ?
	Chemotherapy	Novel agents ?

Disease features as well as different treatment options may either aggravate the negative impact of complex karyotype (listed in the second column) or have a neutral effect (listed in the third column).

CK, complex karyotype, U - CLL, unmutated chronic lymphocytic leukemia, M - CLL, mutated chronic lymphocytic leukemia (adopted from Baliakas et al, Hemasphere 2022)

responses to chemotherapy, and that their disease is typically more aggressive, with only 40% alive after 10-year follow-up (29). The underlying biological mechanisms linking complex karyotype and TP53 aberrations in CLL likely relate to their shared association with genomic instability and genetic damage in CLL cells (27, 29).

The genomic landscape of aberrations in CLL is characterized by heterogeneity and diversity, which can even differ within the same case, defining subclones of the disease. Even so, there seem to be some “order in chaos”, as certain genomic aberrations are more common in some subgroups of CLL – those which are defined by the characteristics of their BcR IG expression (3, 31, 32). This suggests that some connections may exist between specific antigenic triggers and distinct pathways of genomic evolution in CLL. The observed phenomenon also applies to CK, as high CK is often accompanied by TP53 aberrations and U-CLL, pointing to intense cell proliferation (3, 31).

Essentially, CK also emerged as a potentially independent prognostic factor in CLL. An earlier study showed an association between CK and a shorter time to the first treatment, especially in cases with more than five abnormalities ($p < 0.001$). CK with more than 5 abnormalities retained its significance for the time to the first treatment even in multivariate analysis, along with mutational status of IGHV genes and an advanced clinical stage ($p < 0.05$) (28).

In a large retrospective study on CLL by the ERIC (3), CK was detected in 15% of 5290 patients. Advanced clinical stage and previously mentioned negative prognostic factors were statistically significantly more frequent in these patients than in patients without CK ($p < 0.008$). In addition, shorter overall survival (OS) was found in patients with CK (6.9 years, 2.5–18.2 years, $p < 0.0001$). CK retained its significance regarding shorter OS even in multivariable analysis along with other negative parameters. The value of CK as a prognostic parameter was shown in patients with a normal FISH analysis, because the patients with CK and normal FISH experienced significantly shorter OS compared to patients with a normal FISH and without CK (median OS of 7.88 years vs. median OS of 13.7 years, $p < 0.002$). Patients with CK needed the treatment sooner comparing to those without CK (3).

In a study that included 644 untreated patients with CLL, the correlation between CK and OS was examined. CK was detected in 12.3% of patients, on diagnosis or before treatment, and in those patients, OS appeared shorter than in a group without CK (77 months vs. 115 months $p < 0.0001$). In the same study, the impact of known negative prognostic parameters (TP53 and ATM deletions) and CK on OS was assessed. Patients with both CK and TP53 deletion proved to have shorter OS in comparison with patients who only had TP53 deletion ($p < 0.001$) (4).

The previously cited large ERIC study has also demonstrated survival disparity between patients with

CK regarding the number of chromosomal aberrations, grading them into three subgroups: low-CK (3 aberrations), intermediate-CK (4 aberrations), and high-CK (≥ 5 aberrations). The TP53 dysfunction in patients with low and intermediate CK was associated with unfavorable outcome, whilst in patients with high-CK the unfavorable prognosis was observed even in the absence of TP53. The difference among patients with CK was found in those with +12 and +19, because in those patients the disease displays an indolent clinical course, confirming that patients with +12 and +19 form a distinctive group of CLL patients (3).

The risk of developing Richter transformation in patients with CLL and CK is unknown, but considering the negative prognostic value of CK, this association was assessed as plausible in several studies. A retrospective study that included 540 treatment-naïve patients with CLL revealed that CK was significantly more common in patients who developed Richter syndrome than in those who did not, with a seven-fold higher risk of developing Richter transformation in patients with high-CK (18). The analysis of four studies on ibrutinib-treated patients with CLL showed that in patients with CK this transformation is more probable than in those without CK ($p = 0.008$) (33).

CK has not been evaluated as part of a prognostic index yet, mostly because there is a lack of cytogenetic data for most patients included in such studies. It is unclear whether incorporating CK into the development of prognostic indices could enhance their usefulness, since this has not been explored yet.

PREDICTIVE VALUE OF CK IN CLL

The use of CK in treatment decisioning process poses a challenge because of the potential predictive value of CK, especially in the era of personalized therapy (6). The presence of complex karyotype (CK) has been identified as an unfavorable predictive marker in patients with CLL who undergo chemo(immuno)therapy (CIT) (27, 34, 35). This observation suggests that CK may be associated with a worse prognosis and a lower likelihood of a positive treatment outcome. Since this is so, screening for CK prior to treatment initiation may play a vital role in predicting treatment response and selecting appropriate treatment options for patients with CLL. It is important to acknowledge that the precise role of complex karyotype (CK) as an independent predictor in CLL patients undergoing chemo(immuno)therapy remains unclear (3, 5, 27). This knowledge gap arises partly due to the limited inclusion of comprehensive CK assessments in clinical trials evaluating CLL treatments. Consequently, it is uncertain whether the observed association of CK with poor treatment outcomes is solely due to its own impact, or it results from the co-occurrence of other unfavorable

biomarkers, such as TP53 aberrations and U-CLL. To elucidate the ambiguity concerning the independent predictive value of CK in CLL patients undergoing chemo(immuno)therapy, a series of investigations has been undertaken in the context of clinical trials, which will be mentioned hereinafter. These investigations aim to appraise the clinical utility of CK as a potential predictor of treatment outcomes in CLL patients. Through the evaluation of the impact of CK in conjunction with other adverse biomarkers, these studies strive to provide additional insights into the independent prognostic value of CK and its prospective usefulness in predicting treatment response in CLL patients.

In CLL patients treated with chlorambucil-based regimens as first line therapy, as indicated in a prospective study, the interconnection between CK and shorter OS is clear ($p = 0.004$), in spite of the confounding factors. The worst prognosis, as expected, was noted in patients with both CK and TP53 abnormalities ($p < 0.001$) (34).

Regarding CIT based treatments, in a study that included 34.5% of patients with CK treated with rituximab in combination with fludarabine and cyclophosphamide, shorter progression-free survival (PFS) and OS were observed in comparison with patients without CK ($p = 0.005$ and $p = 0.03$, respectively) (27). Similarly, another research proved a relationship between shorter PFS and OS in patients with CK ($p < 0.001$ and $p = 0.02$, respectively) (35).

Based on the previously mentioned studies, CK appears as a negative predictor in patients who are treated with chemotherapy or immunochemotherapy.

According to the latest guidelines, treatment with targeted therapy, such as BTK inhibitors, is strongly recommended. In relapsed/refractory (R/R) CLL patients treated with ibrutinib-based regimens, Thompson et al. suggest that complex karyotype is a stronger predictor than $\text{del}(17p)$ of inferior outcome (36). The study showed that R/R CLL with CK treated with ibrutinib had a shorter event-free survival (EFS) and overall survival (OS) compared to those without CK. Specifically, the association between the presence of CK and shorter EFS was statistically significant ($p = 0.006$), as was the association with shorter OS ($p = 0.008$). However, co-existence of $\text{del}(17p)$ and CK seemed to have a significant impact on these results, because of 21 patients with CK, 17 had $\text{del}(17p)$ (~80%).

In RESONATE study, a prospective study on previously treated patients with CLL, the prognosis for the patients with CK CLL treated with BTK inhibitors was also one of the subjects. In that research no significant differences in PFS and OS were found in patients with CK in comparison to those without CK (≤ 2 cytogenetic aberrations) in median follow-up of 19 months. On the other hand, in this study, in patients with CK who received ofatumumab, there were significantly lower ORR and PFS compared to those without CK (37). After a long fol-

low-up (44 months) median PFS in patients with CK was 40.8 months, as opposed to those without CK, in whom median PFS was not reached (33).

The study Alliance A041202 analyzed the effects of ibrutinib on CLL with CK in individuals who had not received any prior treatment (38). Interestingly, in this study the existence of initial karyotype complexity did not indicate a greater likelihood of progression or mortality in patients treated with ibrutinib, which leads to speculation on whether baseline CK holds the same biological significance as CK resulting from selective clonal expansion following chemotherapy.

Regarding idelalisib regimens, a study which assessed efficacy of idelalisib with rituximab in relapsed CLL patients with significant comorbidities showed longer median OS in the CK group treated with idelalisib (28.3 months), compared to the patients who did not receive target therapy (9.2 months) (39). But there was no difference between ORR in CK and non-CK groups (81% vs. 89%) in patients who received idelalisib. So, it is worth mentioning that this therapy could overcome the bad prognosis of CK, although with limited evidence.

The predictive impact of CK in venetoclax-based regimens was demonstrated in MURANO study, in R/R patients treated with venetoclax-rituximab or bendamustin-rituximab (40). The researchers divided patients into low-, intermediate-, and high-CK, based on genomic complexity. The patients without CK showed better progression-free survival (PFS) compared to those with low-CK or high-CK status (with hazard ratios of 2.0 and 2.9, respectively), and statistically significant differences were observed (with p -values of 0.025 and 0.0057, respectively). Furthermore, patients who had more genomic aberrations exhibited a tendency towards inferior progression-free survival (PFS) compared to those with fewer abnormalities.

In CLL14 trial, the presence of CK among treatment-naïve patients did not significantly affect the outcome of venetoclax-obinutuzumab (VenG) therapy, with ORR 82.4 and 87.3% for patients with CK and non-CK, respectively. Also, the rates of undetectable minimal residual disease (uMRD) were high in both CK and non-CK groups treated with VenG, and there was no significant difference in OS and PFS between these patients (41).

According to a multicenter study on relapsed/refractory CLL patients treated with acalabrutinib, patients with CK, as well as patients with $\text{del}(17p)$ had significantly shorter PFS (median PFS 36 and 33 months, respectively), compared to the rest of the cohort (median PFS not reached) (42).

In the context of patients with CLL who undergo stem cell transplantation, a group of investigators made a score that predicts an outcome for these patients. The presence of ≥ 5 chromosomal abnormalities was found to be a prognostic indicator of PFS outcomes, suggesting that karyotypic complexity may be an important factor

to consider in CLL patients undergoing stem cell transplantation (43).

To conclude, when it comes to clinical trials with target therapy, there is limited information on the predictive impact of CK because the number of cases included is very small, and as a result, any conclusions drawn from it are uncertain. More studies are necessary to obtain robust findings on the predictive significance of CK. Based on the available evidence, CLL patients with a complex karyotype may be less responsive to certain treatments, such as chemotherapy. Therefore, some experts have suggested that targeted therapies, such as B-cell receptor signaling inhibitors and BCL-2 inhibitors, may be more effective in this patient population. However, the optimal treatment approach for CLL patients with a complex karyotype is still an area of active research and debate, and individualized treatment decisions should be based on several factors, including the patient's age, overall health status, and the presence of other genetic and molecular markers.

THE FUTURE IMPLICATIONS OF CK IN CLL

CK is a newly identified potential biomarker in CLL that appears to have a prognostic value, and even more importantly, a predictive value. The identification and characterization of complex karyotype in CLL has important future implications. It has a potential to improve risk stratification and personalized treatment selection for CLL patients, especially in the age of new therapies. Understanding the specific genetic aberrations that contribute

to a complex karyotype may lead to the development of targeted therapies that can address these abnormalities. Additionally, further research on complex karyotype in CLL may provide insights in the mechanisms of disease progression and therapy resistance. Ultimately, a better understanding of clinical implications of complex karyotype in CLL may lead to improved outcomes for patients with this disease.

CONCLUSION

In order to improve treatment decision-making in patients with CLL along with the TP53 mutation presence, mutational status of IGHV, and FISH analysis, conventional cytogenetics should also be evaluated. Conventional cytogenetics can reveal aberrations that are not detected with FISH analysis and confirm the presence of CK. CK in patients with CLL is associated with the progression of the disease and a worse prognosis. In addition, CLL patients with CK should be closely monitored for the Richter transformation during the follow-up period.

Author contributions: Darko Antic – the conception of the design of the work, preparing the draft of the manuscript, approval of revised version of manuscript. Vojin Vukovic – the conception of design of the work, preparing the draft of the manuscript, approval of revised version of manuscript. Sofija Kozarac, Kristina Tomic-Vujovic, Vladimir Otasevic, Andrej Pesic – preparing the draft of the manuscript. Biljana Mihaljevic – approval of revised version of the manuscript.

REFERENCES

- Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. *Am J Hematol* 2021;96(12):1679–705. doi: 10.1002/ajh.26367
- Senouci A, Smol T, Tricot S, Bakala J, Moulessehouli S, Quilichini B, et al. Cytogenetic landscape in 1012 newly diagnosed chronic lymphocytic leukemia. *Eur J Haematol*. 2019;103(6):607–13. doi: 10.1111/ejh.13329
- Baliakas P, Jeromin S, Iskas M, Puiggros A, Plevova K, Nguyen-Khac F, et al. Cytogenetic complexity in chronic lymphocytic leukemia: Definitions, associations, and clinical impact. *Blood*. 2019;133(11):1205–16. doi: 10.1182/blood-2018-09-873083
- Kruzova L, Schneiderova P, Holzerova M, Vatolikova M, Divoka M, Turcsanyi P, et al. Complex karyotype as a predictor of high-risk chronic lymphocytic leukemia: A single center experience over 12 years. *Leuk Res* 2019; 85:106218. doi: 10.1016/j.leukres.2019.106218
- Chatzikonstantinou T, Demosthenous C, Baliakas P. Biology and Treatment of High-Risk CLL: Significance of Complex Karyotype. *Front Oncol*. 2021; 11:1–6. <https://doi.org/10.3389/fonc.2021.788761>
- Jondreville L, Krzisch D, Chapiro E, Nguyen-Khac F. The complex karyotype and chronic lymphocytic leukemia: prognostic value and diagnostic recommendations. *Am J Hematol*. 2020;95(11):1361–7. doi: 10.1002/ajh.25956
- Strati P, Wang F, Tambaro FP, Thompson PA, Burger JA, Jain N, et al. The landscape of genetic mutations in patients with chronic lymphocytic leukaemia and complex karyotype. *Br J Haematol*. 2019;187(1):e1–4. doi: 10.1111/bjh.16117
- Gherge M, Lazar AM, Mutuleanu MD, Bordea CI, Ionescu S, Mihaila RI, et al. Evaluating Cardiotoxicity in Breast Cancer Patients Treated with HER2 Inhibitors: Could a Combination of Radionuclide Ventriculography and Cardiac Biomarkers Predict the Cardiac Impact? *Cancers (Basel)*. 2023;15(1). doi: 10.3390/cancers15010207.
- Baliakas P, Iskas M, Gardiner A, Davis Z, Plevova K, Nguyen-Khac F, et al. Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: A systematic reappraisal of classic cytogenetic data. *Am J Hematol*. 2014;89(3):249–55. doi: 10.1002/ajh.23618
- Eichhorst B, Hallek M. Prognostication of chronic lymphocytic leukemia in the era of new agents. *Hematology Am Soc Hematol Educ Program*. 2016; 2016(1): 149–155. doi: [10.1182/asheducation-2016.1.149](https://doi.org/10.1182/asheducation-2016.1.149)
- Mato AR, Roeker LE, Lamanna N, Allan JN, Leslie L, Pagel JM, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020;136(10):1134–43. doi: 10.1182/asheducation-2016.1.149
- Pabon CM, Brander DM, Stewart T, Weinberg JB. Novel Prognostic Markers in Previously Treated Chronic Lymphocytic Leukemia. *Blood*. 2021; 138:4688.
- Edelmann J, Malcikova J, Riches J. Opinion: What defines high-risk CLL in the post-chemoimmunotherapy era?, *Front Oncol*. 2023; 13:379. <https://doi.org/10.3389/fonc.2023.1106579>

14. Tosic N, Ugrin M, Marjanovic I, Kostic T, Vukovic V, Tomic K, et al. Expression of BCL11A in chronic lymphocytic leukaemia. *Int J Lab Hematol.* 2023;45(1):64-71. doi: 10.1111/ijlh.13969
15. Mitrovic Ajtic O, Suboticki T, Diklic M, Djikic D, Vukotic M, Dragojevic T, et al. Regulation of S100As Expression by Inflammatory Cytokines in Chronic Lymphocytic Leukemia. *Int J Mol Sci.* 2022;23(13):6952. doi: 10.3390/ijms23136952
16. Baliakas P, Espinet B, Mellink C, Jarosova M, Athanasiadou A, Ghia P, et al. Cytogenetics in Chronic Lymphocytic Leukemia: ERIC Perspectives and Recommendations. *HemaSphere.* 2022;6(4): E707. doi: 10.1097/HS9.0000000000000707
17. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analyzed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. *Leukemia* 2007;21(12):2442–51. doi: 10.1038/sj.leu.2404935
18. Dicker F, Schnittger S, Haferlach T, Kern W, Schoch C. Immunostimulatory oligonucleotide-induced metaphase cytogenetics detect chromosomal aberrations in 80% of CLL patients: a study of 132 CLL cases with correlation to FISH, IgVH status, and CD38 expression. *Blood* 2006;108(9):3152–61. doi: 10.1182/blood-2006-02-005322
19. Visentin A, Bonaldi L, Rigolin GM, Mauro FR, Martines A, Frezzato F, et al. The complex karyotype landscape in chronic lymphocytic leukemia allows the refinement of the risk of Richter syndrome transformation. *Haematologica* 2022;107(4):868. doi: 10.3324/haematol.2021.278304
20. Kittai AS, Miller C, Goldstein D, Huang Y, Abruzzo LV, Beckwith K, et al. The impact of increasing karyotypic complexity and evolution on survival in patients with CLL treated with ibrutinib. *Blood.* 2021;138(23):2372–82. doi: 10.1182/blood.2020010536
21. Baliakas P, Puiggros A, Xochelli A, Sutton LA, Nguyen-Khac F, Gardiner A, et al. Additional trisomies amongst patients with chronic lymphocytic leukemia carrying trisomy 12: the accompanying chromosome makes a difference. *Haematologica.* 2016;101(7): e299. doi: 10.3324/haematol.2015.140202
22. Thomay K, Fedder C, Hofmann W, Kreipe H, Stadler M, Titgemeyer J, et al. Telomere shortening, TP53 mutations and deletions in chronic lymphocytic leukemia result in increased chromosomal instability and breakpoint clustering in heterochromatic regions. *Ann Hem.* 2017; 96:1493–500. doi: 10.1007/s00277-017-3055-1
23. Zucker M, Abruzzo L, Herling C, Barron L, Keating M, Abrams Z, et al. Inferring clonal heterogeneity in cancer using SNP arrays and whole genome sequencing. *Bioinformatics.* 2019;35(17):2924–31. doi: 10.1093/bioinformatics/btz057
24. Guièze R, Robbe P, Clifford R, De Guibert S, Pereira B, Timbs A, et al. Presence of multiple recurrent mutations confers poor trial outcome of relapsed/refractory CLL. *Blood.* 2015;126(18):2110–7. doi: 10.1182/blood-2015-05-647578
25. Koczkodaj D, Poppek-Marciniak S, Zmorzyński S, Wąsik-Szczepanek E, Filip AA. Examination of clonal evolution in chronic lymphocytic leukemia. *Med Oncol.* 2019; 36:1–4. doi: 10.1007/s12032-019-1300-2
26. Cavallari M, Cavazzini F, Bardi A, Volta E, Melandri A, Tammiso E, et al. Biological significance and prognostic/predictive impact of complex karyotype in chronic lymphocytic leukemia. *Oncotarget.* 2018;9(76):34398. <https://doi.org/10.18632/oncotarget.26146>
27. Le Bris Y, Struski S, Guièze R, Rouvellat C, Prade N, Troussard X, et al. Major prognostic value of complex karyotype in addition to TP53 and IGHV mutational status in first-line chronic lymphocytic leukemia. *Hematol Oncol* 2017;35(4):664–70. doi: 10.1002/hon.2349
28. Baliakas P, Iskas M, Gardiner A, Davis Z, Plevova K, Nguyen-Khac F, et al. Chromosomal translocations and karyotype complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data. *Am J Hematol* 2014;89(3):249–55. doi: 10.1002/ajh.23618
29. Visentin A, Bonaldi L, Rigolin GM, Mauro FR, Martines A, Frezzato F, et al. The combination of complex karyotype subtypes and IGHV mutational status identifies new prognostic and predictive groups in chronic lymphocytic leukaemia. *British journal of cancer.* 2019;121(2):150–6. doi: 10.1038/s41416-019-0502-x
30. Karan-Djurasevic T, Palibrk V, Kostic T, Spasovski V, Nikcevic G, Srzentic S, et al. Mutational status and gene repertoire of IGHV-IGHD-IGHJ rearrangements in Serbian patients with chronic lymphocytic leukemia. *Clin Lymphoma Myeloma Leuk.* 2012;12(4):252–60. <https://doi.org/10.1016/j.clml.2012.03.005>
31. Sutton LA, Young E, Baliakas P, Hadzidimitriou A, Moysiadis T, Plevova K, et al. Different spectra of recurrent gene mutations in subsets of chronic lymphocytic leukemia harboring stereotyped B-cell receptors. *Haematologica.* 2016;101(8):959. doi: 10.3324/haematol.2016.141812
32. Agathangelidis A, Chatzidimitriou A, Gemenetzi K, Giudicelli V, Karypidou M, Plevova K, et al. Higher-order connections between stereotyped subsets: implications for improved patient classification in CLL. *Blood.* 2021;137(10):1365–76. doi: 10.1182/blood.2020007039
33. Byrd JC, Hillmen P, O'Brien S, Barrientos JC, Reddy NM, Coutre S, et al. Long-term follow-up of the RESONATE phase 3 trial of ibrutinib vs ofatumumab. *Blood* 2019;133(19):2031–42. doi: 10.1182/blood-2018-08-870238
34. Herling CD, Klauwünzer M, Rocha CK, Altmüller J, Thiele H, Bahlo J, et al. Complex karyotypes and KRAS and POT1 mutations impact outcome in CLL after chlorambucil-based chemotherapy or chemoimmunotherapy. *Blood* 2016;128(3):395–404. doi: 10.1182/blood-2016-01-691550
35. Badoux XC, Keating MJ, Wang X, O'Brien SM, Ferrajoli A, Faderl S, et al. Fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy is highly effective treatment for relapsed patients with CLL. *Blood* 2011;117(11):3016–24. doi: 10.1182/blood-2010-08-304683
36. Thompson PA, O'Brien SM, Wierda WG, Ferrajoli A, Stingo F, Smith SC, et al. Complex karyotype is a stronger predictor than del (17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. *Cancer.* 2015;121(20):3612–21. doi: 10.1002/cncr.29566
37. Brown JR, Hillmen P, O'Brien S, Barrientos JC, Reddy NM, Coutre SE, et al. Extended follow-up and impact of high-risk prognostic factors from the phase 3 RESONATE study in patients with previously treated CLL/SLL. *Leukemia* 2018;32(1):83–91. doi: 10.1038/leu.2017.175
38. Woyach JA, Ruppert AS, Heerema NA, Zhao W, Booth AM, Ding W, et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. *New Engl J Med.* 2018;379(26):2517–28. doi: 10.1056/NEJMoa1812836
39. Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *New Engl J Med.* 2014;370(11):997–1007. doi: 10.1056/NEJMoa1315226
40. Kater AP, Wu JQ, Kipps T, Eichhorst B, Hillmen P, D'rozario J, et al. Venetoclax plus rituximab in relapsed chronic lymphocytic leukemia: 4-year results and evaluation of impact of genomic complexity and gene mutations from the MURANO phase III study. *J Clin Oncol.* 2020;38(34):4042. doi: 10.1200/JCO.20.00948
41. Al-Sawaf O, Lilienweiss E, Bahlo J, Robrecht S, Fink AM, Patz M, et al. High efficacy of venetoclax plus obinutuzumab in patients with complex karyotype and chronic lymphocytic leukemia. *Blood.* 2020;135(11):866–70. doi: 10.1182/blood.2019003451
42. Byrd JC, Wierda WG, Schuh A, Devreux S, Chaves JM, Brown JR, et al. Acalabrutinib monotherapy in patients with relapsed/refractory chronic lymphocytic leukemia: updated phase 2 results. *Blood* 2020;135(15):1204–13. doi: 10.1182/blood.2018884940
43. Kim HT, Ahn KW, Hu ZH, Davids MS, Volpe VO, Antin JH, et al. Prognostic Score and Cytogenetic Risk Classification for Chronic Lymphocytic Leukemia Patients: Center for International Blood and Marrow Transplant Research Report Prognostic Score and Cytogenetic Risk in CLL after HCT. *Clin Cancer Res.* 2019;25(16):5143–55

KOMPLEKSNI KARIOTIP U HRONIČNOJ LIMFOCITNOJ LEUKEMIJI

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Sažetak

Hronična limfocitna leukemija (HLL) je genetski heterogeno oboljenje u kojem se metodama konvencionalne ili molekularne citogenetike registruju hromozomske aberacije u više od 80% pacijenata. Kompleksni kariotip (CK) se definiše kao prisustvo ≥ 3 strukturne ili numeričke aberacije u istom klonu maligne HLL ćelije, i smatra se mogućim prognostičkim parametrom u HLL. Detekcija

CK kod pacijenata sa HLL potencijalno može uticati na prognozu i odabir terapijskog modaliteta, uzimajući u obzir povezanost CK sa lošijom prognozom i progresijom HLL, kao i sa povećanim rizikom od razvoja Rihterove transformacije. U ovom preglednom radu biće razmotrena kompleksnost analize kariotipa u HLL i njegov prognostički i klinički značaj.

Cljučne reči: hronična limfocitna leukemija, kompleksni kariotip, hromozomske aberacije

Primljen: 13.06.2023. | **Revizija:** 30.06.2023. | **Prihvaćen:** 07.07.2023

Medicinska istaživanja 2023; 56(3):77-85