CASE REPORT

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Rapid progression to Richter's syndrome in a patient with chronic lymphocytic leukemia and near-triploid karyotype

Brza progresija hronične limfocitne leukemije u Rihterov sindrom kod bolesnika sa kariotipom blizu triploidnog broja hromozoma

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Abstract

Introduction. The presence of aneuploidy in patients diagnosed with chronic lymphocytic leukemia (CLL), except trisomy 12, is considered quite uncommon. Hyperdiploidy or near-tetraploidy (occurring in 1–3% of all CLL patients) usually confer a poor prognosis. **Case report.** We report a patient in a progressive phase of CLL with near–triploid karyotype. The prognosis of the disease was more precisely determined by applying the cytogenetic analysis of the karyotype and was complemented with molecular methods and pathohistological examination. The complex karyotype was accompanied by the *TP53*, *C-MYC*, and *IGH* gene disruptions, the most probable cause of rapid evolution into Richter's syndrome. **Conclusion.** The use of comprehensive contemporary diagnostic techniques is highly recommended in patients who are in the progressive phase of CLL, primarily for the adequate choice of management strategy. The presented case confirms that aneuploidy in CLL patients indicates poor prognosis, which is in accordance with previous publications reporting on cases of CLL patients with aneuploidy.

Key words:
abnormal karyotype; chronic lymphocytic leukemia; disease progression; karyotyping; richter's syndrome.

Apstrakt

Uvod. Prisustvo aneuploidije kod bolesnika sa dijagnozom hronične limfocitne leukemije (HLL), sa izuzetkom trizomije 12, smatra se retkom pojavom. Pojava hiperdiploidnog ili kariotipa blizu tetraploidnog broja hromozoma (koji se javlja kod 1–3% svih bolesnika sa HLL) smatra se lošim prognostičkim parametrom. **Prikaz bolesnika.** Prikazan je bolesnik u uznapredovaloj fazi HLL sa kariotipom blizu triploidnog broja hromozoma. Prognoza bolesti je preciznije određena citogenetičkom analizom kariotipa bolesnika, i dopunjena molekularnim metodama i patohistološkom analizom. Otkriveno je prisustvo kompleksnog kariotipa udruženog sa poremećajima u genima *TP53, C-MYC* i *IGH*, što je najverovatnije bio uzrok brze progresije u Rihterov sindrom. **Zaključak.** Primena savremenih dijagnostičkih metoda veoma je značajna kod bolesnika u uznapredovaloj fazi HLL, prvenstveno zbog adekvatnog terapijskog pristupa. Prikazani slučaj ukazuje da je prisustvo aneuploidije kod bolesnika sa HLL loš prognostički znak, što je u saglasnosti sa prethodno publikovanim prikazima bolesnika sa HLL i sa aneuploidijom u kariotipu.

Ključne reči:
kariotip, abnormalni; hronična limfocitna leukemija; bolest, progresija; kariotip, određvanje; rihterov sindrom.

Introduction

Chromosomal aberrations in chronic lymphocytic leukemia (CLL) are of key importance for predicting disease outcomes and are often used in therapeutic decisions [1, 2]. These alterations, including trisomy (TS) 12 (+12) and deletion of chromosomes 13q14 (13q-), 11q22 (11q-), and 17p13 (17p-), are detected in more than 80% of the CLL cases by fluorescence *in situ* hybridization (FISH) technique [1]. However, FISH provides information only

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about chromosomal regions covered by the probes used, leaving the majority of the present karyotype changes undetectable.

In recent years, various studies revealed the prognostic impact of chromosomal abnormalities (CAs), which are not covered with FISH probes [3–5]. The International Workshop on CLL [6] proposed conventional karyotype analysis be performed before first-line therapy and any subsequent treatment due to the frequent gain of additional CAs during the course of the disease.

Complex karyotype with three or more abnormalities is present in 14–34% of untreated CLL patients and confers a worse disease outcome [5, 7, 8]. The estimation of karyotype complexity at the time of CLL diagnosis is crucial since its presence has been linked to the evolution of Richter's syndrome (RS) [9]. The most frequent aberrations in complex karyotypes include structural changes in chromosomes 11, 13, 14, and 17. On the contrary, the occurrence of numerical changes (except TS 12) is extremely rare and, when present, can be seen within hyperdiploid or near-tetraploid karyotypes in 1–3% of all CLL cases [4, 10–13].

Herein, we report a CLL patient with a complex, near-triploid karyotype in the progressive phase of CLL, who rapidly evolved to RS.

Case report

A 71-year-old female presented with a six-month history of fatigue, sweating, weight loss, and enlargement of tonsils, peripheral lymph nodes, and spleen. Laboratory findings revealed the following: hemoglobin 108 g/L [reference range (RR) 120–160 g/L], white blood cells 10.7x10^9/L (RR 3.6–10.0 x10^9/L), with neutrophils 12% (RR 42–75%), lymphocytes 21% (RR 20–51%), atypical lymphoid cells 37%, monocytes 27% (RR 2–10%), platelets 202 x10^9/L (RR 150–450 x10^9/L), elevated lactate dehydrogenase (LDH) 735 IU/L (RR 220–460 IU/L), β2 microglobulin 7.5 mg/L (RR 970–2,640 mg/L) and C-reactive protein 1.9 mg/L (RR 0.0–3.0 mg/L). Flow cytometry confirmed score 5 for CLL with CD38 and CD49d positivity [$CD19^+$, $CD20^{low}$, $CD21^{low}$, $CD22^{low}$, $CD2^{interm}$, $CD24^{intermed}$, $CD5^{bright}$, $CD43^{low}$, $CD49d^{low}$, $CD38^*$(*the pattern of CD38 expression profile was characterized as bimodal, with the concomitant presence of one population expressing high levels of CD38 and a second population completely negative), $FMC7^-$, $CD79b^-$].

Conventional cytogenetic analysis was performed on chromosome metaphases prepared from peripheral blood lymphocytes cultured for 72 hrs with lipopolysaccharide using the standard technique. Giemsa-banded metaphases were analyzed, and the findings were reported according to the International System for Human Cytogenetic Nomenclature in 2013 [12].

FISH analysis for common cytogenetic abnormalities associated with CLL was performed on interphase nuclei obtained from the culture of peripheral blood samples and tissue sections prepared from a formalin-fixed, paraffin-embedded tonsilar biopsy. A panel of probes designed to detect TS 12 and deletions of 13q14.3, 17p13.1, and 11q22.3, according to the manufacturer's instructions (Vysis/Abbott Laboratories, Des Plaines, IL), were used. In order to assess the immunoglobulin heavy locus (*IGH*) gene and *C-MYC* proto-oncogene, FISH analysis was performed using dual-color break-apart probes (Vysis/Abbott Laboratories).

For tumor protein 53 (*TP53*) mutational analysis, DNA was isolated from formalin-fixed, paraffin-embedded bone marrow samples using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The *TP53* mutational status was determined by polymerase chain reaction amplification of coding exons 4–10 and flanking intronic regions, as recommended in Pospisilova et al. [13], followed by direct Sanger sequencing (forward and reverse strand) with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on 3130 Genetic Analyzer (Applied Biosystems, USA). The results were interpreted by GLASS software, a web-based Sanger sequence trace viewer, editor, aligner and variant caller, and the locus-specific International Agency for Research on Cancer (IARC) database.

The computerized tomography scan showed an enlargement of lymph nodes from 14 mm in the mediastinal area and 21 mm in the retroperitoneum to 29 mm in the axillar region. The liver was normal in size and appearance; the spleen was enlarged (150 mm) with the presence of homogenous bilateral pleural effusion. Bone marrow core biopsy revealed diffuse infiltration with small lymphocytes (80%). Due to tonsilar enlargement, a tonsillectomy was performed. Histopathological evaluation of the tonsils, followed by immunohistochemical analysis, revealed signs of initial progression of small lymphocytic lymphoma (SLL)/CLL into diffuse large B-cell lymphoma (DLBCL), the occurrence of single large cells without sheets, with the phenotype PAX-5^+, CD79α$^+$, $CD20^+$, $CD3^-$, $CD5^+$, $CD23^+$, $CD43^+$, Cyclin $D1^-$, $SOX11^-$, bcl-2^+, bcl-6^-, $CD10^-$, $CD38^-$, $CD30^-$, $CD15^-$, $MUM1^-$, C-MYC^-, $ZAP70^-$, $p53^+$, Ki-67^+ in 30-40% of them, approximately three mitoses per proliferation center (Figure 1). Tonsilar tissue FISH analysis confirmed the result obtained from peripheral blood, with an additional chromosome copy number. Finally, the patient was diagnosed with CLL Rai stage III and was initially treated with a high dose of methylprednisolone, followed by rituximab, fludarabine, and cyclophosphamide (R-FC) lite protocol, which she did not tolerate. Febrile neutropenia with prolonged recovery developed, and after improvement, treatment was continued with cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisolone (CHOP) protocol, again with poor response and further lymph node progression. Due to right popliteal phlebothrombosis, anticoagulant treatment was commenced. Because of the substantial toxicity of chemotherapy, additional palliative treatment and extra methylprednisolone cycles were applied. Unfortunately, 11 months after diagnosis, the patient experienced intestinal complications with positive *Clostridium difficile* enterocolitis, leading to the fatal outcome.

Conventional cytogenetic analysis was performed on 20 chromosome metaphases from a peripheral blood sample,

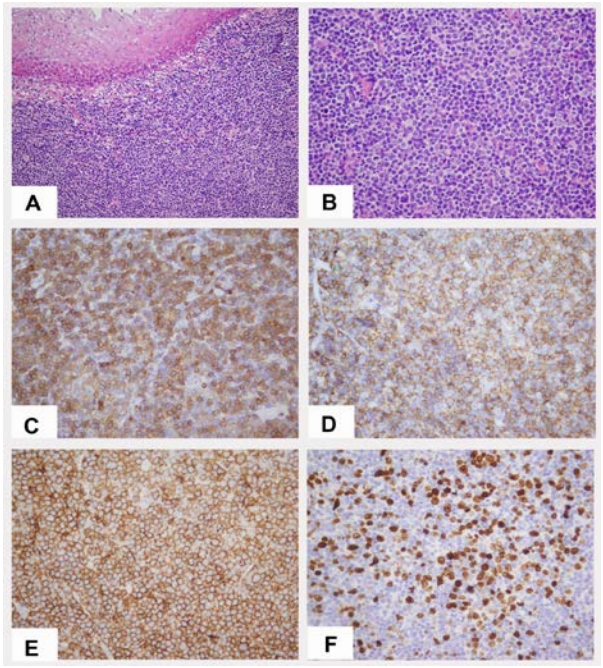

Fig. 1 − Biopsy of tonsilar tumor: A) Increased number of large single cells with immunoblastic features and expanded proliferation centers [hematoxylin and eosin (HE), ×100]; B) Approximately three mitoses per proliferation center (HE, ×400); C-F) Immunohistochemical features of tumor cells (streptavidin-biotin, ×400): C) CD79α staining; D) CD5 staining; E) CD23 staining; F) Ki-67 staining highlights high proliferation rate of the cells in the proliferation centers.

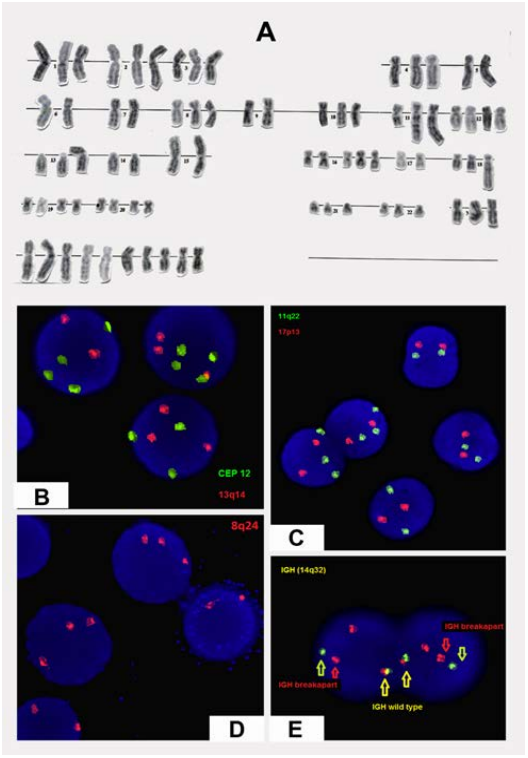

Fig. 2 − A) Near-triploid karyotype with complex aberrations from peripheral blood; B) Trisomy and tetrasomy of CEP12 accompanied with trisomy of 13q14; C) Trisomy of 11q22 with disomy of 17p13; D) Trisomy of 8q24 (*C-MYC*); E) Rearrangement of 14q32 (*IGH*) showing an extra red signal of the 3'*IGH*.

revealing a near-triploid female karyotype with multiple numerical and structural changes in 13 out of 20 metaphases, described as: 70-77,XXX,+1,+2,+3,+4,+8,der(9)add(9)(p24),+10,der(11)add(11)(q25),+der(11)add(11)(q25),+12,+12,+der(13)add(13)(p11),der(15)add(15)(p11),+der(15)add(15)(p11),+16,+16,+16,der(18)add(18)(q22),+19,+19,+20,+20,+21,+22,+10mar[cp13]/46,XX [7] (Figure 2A).

FISH analysis of peripheral blood and paraffin-embedded tonsilar and bone marrow tissue, using the CLL panel, revealed three copies of chromosomes 11q22.3, 8q24, and 13q14.3 in 30%, 50%, and 70% of nuclei, respectively. Moreover, three and four copies of chromosome enumeration probes (CEP) 12 were spotted in a total of 70% of nuclei, while the rearrangement of the *IGH* gene with an extra copy of the 3'*IGH* signal was present in 30% of nuclei. These findings were presented in Figures 2B–E and described as: nuc ish [(D13S319,D12Z3)x(380/200)]/nuc ish (D13S319x3,D12Z3x4)[60/200] (Figure 2B); nuc ish [ataxia telangiectasia mutated (ATM)x3,*TP53*x*2*][60/200] (Figure 2C); nuc ish(*C-MYC* x 3)(5'*C-MYC* con 3'*C-MYC* x3)[100/200] (Figure 2D); nuc ish(3'*IGH*x3,5'*IGH*x2)(3'*IGH* con 5'*IGH*x1)[60/200] (Figure 2E). *TP53* mutational analysis revealed frameshift mutation in exon 6 [c.626_627delGA (p.R209fs*6)].

Discussion

The incidence rate of progression of CLL to DLBCL in newly diagnosed patients is relatively rare; it usually occurs in 0.5–1.1% of patients and develops 1.8–4 years after the initial diagnosis, depending on the duration of clinical follow-up, patient population studied, and diagnostic criteria used to define it [4, 14, 15]. Some of the biological factors predictive of RS development include expression of CD38 and CD49, inactivation of *TP53* or *C-MYC* abnormalities, as well as genomic complexity, which has been considered an adverse prognostic indicator [16–18].

Our patient was diagnosed with SLL/CLL, showing splenomegaly, more than three enlarged lymph node regions, and elevated serum LDH and β2 microglobulin levels at presentation. The presentation of highly progressive disease was established by other diagnostic methods, including immunophenotype (CD38 and CD49d positivity), pointing out that the patient had an increased risk of both short survival and clonal evolution [17, 18].

The rapid disease evolution and the patient's resistance to chemotherapy coincided with the presence of a near-triploid karyotype. The FISH result revealed TS of the 13q14 region and *C-MYC* gene as the most prevalent aberrations,

followed by TS of 11q, CEP12, and tetrasomy of chromosome 12. The karyotype analysis defined trisomic signals seen by FISH more closely: two signals of the 11q22 region marked two derivative chromosomes 11, one of the three signals for 13q14 belonged to one derivative chromosome 13, while three and four signals for 8q24 and CEP12, respectively, presented simple copy number changes of the whole chromosomes 8 and 12. In addition, multiple structural and numerical changes of chromosomes not covered with FISH probes were observed as well.

However, the karyotype itself gave us no information about the sequence of events leading to its development. A relatively short period of time (6 months) from the first disease symptoms and rapid progression to RS can be explained by the early acquisition of some of the high-risk gene mutations, which would favor chromosomal instability and the development of additional chromosomal changes. Indeed, mutational analysis of the *TP53* gene was positive for the frameshift mutation in exon 6, while FISH for *IGH* and *C-MYC* genes showed positivity for both rearrangements. It is worth noting that our findings were in contrast with the recent report of Rossi et al. [19], who found that TS 12 and 17p aberrations seemed mutually exclusive.

In our report, the presence of near-triploid karyotype was designated as an uncommon and very rare event in both CLL and RS. However, in the context of complex karyotype, our results are consistent with similar published cases showing karyotype complexity with *TP53* and/or *C-MYC* rearrangements as particularly adverse prognostic indicators, often seen in CLL patients with the evolution to RS [4, 7].

Conclusion

We can point out that karyotype analysis revealed infrequent cytogenetic aberrations in CLL, contributing to the identification of the CLL patient with a highly aggressive clinical course. The combination of morphologic and immunohistochemical analyses, together with the incorporation of conventional cytogenetics in CLL diagnostics, could provide complementary information to FISH and mutation analyses in defining high-risk patients and facilitating therapy strategy.

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