INTRODUCTION

Utilizing interphase fluorescence in situ hybridization (FISH), investigators have detected chromosomal aberrations in over 80% of cases of chronic lymphocytic leukemia (CLL). These aberrations commonly involve chromosomes 11, 12, 13, and 17. Several retrospective studies suggest that the given chromosomal abnormalities are correlated with various disease parameters (Dohner et al., 2000; Wiktor et al., 2006). The general picture is dominated by unbalanced changes, mainly with deletions of well-definable chromosomal regions. The abnormalities which seem to be associated with poor prognosis, e.g., +12, del(17)(p13.1), and del(11)(q22.3), are ones which have been shown to be secondary events, i.e. present in some of the malignant B cells and developing at a later stage after the initiating event. On the other hand, deletion of one or more genes on the long arm of chromosome 13 is thought to be critical as an early event in CLL (Mehes, 2005). Moreover, 13q deletion has been linked with favorable prognosis, whereas trisomy 12 has been associated with higher proliferative activity and short survival. In most previous studies, a low percent of coexistence of trisomy 12 and deletions of 13q14 was observed (Peterson et al., 1992; Avet-Loiseau et al., 1996; Mould et al., 1996; Garcia-Marco et al., 1997; Hogan et al., 1999).

We here describe two patients in whom interphase FISH analysis showed trisomy 12 and del(13)(q14.3) occurring in the same clone. These abnormalities are rarely seen together and the prognostic relevance of their coexistence is still unclear. According to some data, a probable adverse prognosis for this group of patients is suggested. Our patients have been in a stable phase of the disease for more than one year since the given abnormalities were documented in their karyotypes. Further study is necessary to determine the prognostic significance of coexistence of these abnormalities in CLL patients.

MATERIALS AND METHODS

Case reports

Patient 1

A 78-year-old man presented with fatigue and nocturnal sweating three years ago. The blood count showed: hemoglobin 13.6 g/dl, platelets 187 x 10^9/l, and white blood cells 60 x 10^9/l. The differential count showed neutrophils 6%, lymphocytes 92%, and monocytes 2%. Diagnosis of CLL was confirmed by immunophenotypic analysis performed on peripheral blood mononuclear cells, which demonstrated the expression of mature B-cell markers...
(CD19, CD22), the coexpression of CD5 and CD23, and the absence of sIg, CD 79b, FMC7, and CD38 expression.

The bone marrow aspirate was hypercellular with 90% infiltration by small lymphocytes. Trephine biopsy showed a diffuse pattern of infiltration. According to the Rai staging system, the patient was considered to be in stage I. At his last check-up, he was without signs of progression in physical examination, but with increase of WBC up to 92 x 10⁹/l.

**Patient 2**

A 39-year-old man presented with cervical, axillary, and inguinal lymphadenopathies up to 1.5 cm, without palpable organomegaly. The blood count showed: hemoglobin 15.6 g/dl, platelets 181 x 10⁹/l, and white blood cells 15.9 x 10⁹/l. The differential count showed neutrophils 22%, lymphocytes 71%, and monocytes 7%. Initial ultrasonography of the abdomen showed no enlargement of the liver and spleen without retroperitoneal lymphadenopathy.

Diagnosis of CLL was confirmed by immunophenotypic analysis performed on peripheral blood mononuclear cells, which demonstrated the expression of mature B-cell markers (CD19, CD22), the coexpression of CD5, CD23, sIg, CD 79b, and FMC7, and the absence of CD38 expression.

The bone marrow aspirate was hypercellular with 84% infiltration by small lymphocytes. Trephine biopsy showed an interstitial pattern of infiltration. At the time of diagnosis, the patient was considered to be in stage I according to the Rai system. At his last check-up, he was with general peripheral lymphadenopathy up to 3 cm in diameter, palpable splenomegaly and with increase of WBC up to 60 x 10⁹/l.

**Fluorescence in situ hybridization (FISH)**

The CLL FISH Probe Panel, consisting of Sets 1 and 2, was used (Vysis, Downers Grove, IL). Probe Set 1 contains the SpectrumOrange LSI p53 probe (17p13.1) and the SpectrumGreen LSI ATM (11q22.3) probe. Probe Set 2 is composed of the SpectrumOrange D13S319 (13q14.3) probe, the SpectrumAqua LSI13q34 (13q34) probe, and the SpectrumGreen CEP 12 probe, which contains the D12Z3 alpha satellite sequence located at the centromere of chromosome 12. The FISH procedure was performed on peripheral blood cell chromosome preparations according to the manufacturer's instructions.

Two hundred nuclei were analyzed for each probe. The cutoff value for positivity was >10% for monosomies of D13S319, 13q34, ATM, and p53; and >10% for trisomy 12.

Slides were examined using a Zeiss fluorescent microscope.

**RESULTS**

In patient No.1, interphase FISH analysis revealed trisomy 12 accompanied by 13q14.3 deletion in 68% of the nuclei scored (Fig. 1a). A clone with deletion of 13q14.3 but without trisomy 12 was observed in another 20% of the nuclei (Fig. 1b), and two signals for each probe (normal FISH pattern) were detected in the remaining 12% of the cells (Fig. 1c). In patient No. 2, the distribution of aberrant and normal clones were similar: both aberrations (trisomy 12 and 13q14.3 deletion) were noticed in 60% of the nuclei, deletion of 13q14.3 as a single aberration was noticed in 25% of the nuclei, and the normal FISH pattern for chromosome 12 and the 13.14.3 region was noticed in the remaining 15% of the cells. Since the same FISH probe set was used for both patients, the results for patient No. 2 are not presented in the figure.

Analysis by FISH with LSI ATM/p53 probes showed a normal FISH pattern in all analyzed cells of both patients, suggesting that deletions of the 11q22.3 and 17p13.1 regions were not present in their karyotypes (data not shown).

**DISCUSSION**

The first chromosomal analyses performed on CLL patients identified trisomy 12 as the most frequent chromosomal abnormality, but in recent FISH studies deletions involving 13q turned out to be the
most common aberrations (Peterson et al., 1992; Avet-Loiseau et al., 1996; Mould et al., 1996; Garcia-Marco et al., 1997; Hogan et al., 1999).

It is speculated that +12 and del(13q)(14.3) have two independent molecular genetic pathways and that consequently these abnormalities rarely coexist in the same pathologic clone. However, in relatively high frequencies of cases (up to 17%) with both aberrations were found in two studies (Navarro et al., 1998; Chena et al., 2003).

Using FISH, we found the coexistence of trisomy 12 and 13q14.3 deletion in the karyotypes of two patients with CLL diagnosis. The finding that trisomy 12 is less present than del(13q)(14.3) in malignant clones indicates that +12 could not be the initial event in leukemogenesis and is rather a result of karyotypic evolution in our patients.

The biological role of trisomy 12 in pathogenesis of CLL has not yet been clarified. It is speculated that a duplicated 12q13-q21.2 region may contain candidate oncogene(s) with a pathogenic role in CLL.

The clinical significance of trisomy 12 and 13q14 aberrations was first reported at the First and Second IWCCLL, where patients with trisomy 12 were shown to have the shortest survival times among patients with single chromosomal abnormalities. On the other hand, patients with structural abnormalities of chromosome 13 seemed to have a more favorable prognosis, exhibiting survival probabilities similar to those with a normal karyotype (Juliusson et al., 1990; Juliusson et al., 1991).

It was discovered that the deleted 13q14.3 region harbors a set of non-protein-coding micro RNA genes (Calin et al., 2004). These genes express short (approx. 20-30 bp) functional RNA molecules that would be capable of regulating distant sites of the genome by RNA interference. Mitochondrial RNA originating from the 13q14 locus could play an important role in the regulation of cellular activation, and altered balance of this control mechanism therefore might slow down progression of the disease (Mehes, 2005).

Long-term clinical studies point to an unfavorable nature of trisomy 12, although not all data could confirm a difference of survival rates compared to patients with normal karyotype (Mehes, 2005). Furthermore, patients exhibiting this aberration are frequently associated with advanced Rai stages, CD38 expression, and atypical morphology (Athanasiadou et al., 2006).

The prognostic relevance of the coexistence of trisomy 12 and deletion 13q14.3 is still unclear and one study (Chena et al., 2003) suggests a probable adverse prognosis for this group of patients.
To the contrary, our results show that both of our patients are in a stable phase of the disease (Rai I), without CD38 expression and atypical morphology. Undoubtedly, clinical and molecular data of more cases are needed to determine the prognostic significance of coexistence of these abnormalities in CLL patients.

REFERENCES


ИСТОВРЕМЕНО ПРИСУСТВО ТРИЗОМИЈЕ 12 И DEL(13)(Q14.3) КОД ДВА ПАЦИЈЕНТА СА ХРОНИЧНОМ ЛИМФОЦИТНОМ ЛЕУКЕМИЈОМ

МАРИЈА ДЕНЧИЋ-ФЕКЕТЕ¹, Д. АНТИЋ¹, САЊА ДАВИДОВИЋ-МРСИЋ², ИВАНА ФРАНИЋ², НАДА КРАГУЉАЦ-КУРТОВИЋ¹, ЈЕЛЕНА БИЛА¹ и И. ЕЛЕЗОВИЋ¹

¹Институт за хематологију, Клинички центар Србије, 11000 Београд, Србија
²Клинички институт за лабораторијску дијагностику, Клинички центар “Ребро”, 10000 Загреб, Хрватска

Презентујемо два пацијента са дијагнозом хроничне лимфоцитне леукемије (CLL), код којих је анализом интерфазне флуоресцентне in situ хибридизације (FISH) откривено присуство тризомије 12 and del(13)(q14.3) у истом патолошком клону. Ове аномалности се ретко виђају заједно, а прогностичка значајност њихове коегзистенције је још увек нејасна. Према неким литературним подацима, за ову групу пацијената се предвиђа лоша прогноза и брза прогресија болести. Супротно томе, наши пацијенти се налазе у стабилној фази болести, већ дуже од годину дана од како су ове аномалности детektоване у њиховом кариотипу. Даље пратење ових пацијената је неопходно у циљу утврђивања прогностичке значајности истовременог присуства ових аберација код CLL пацијената.