

t(14;18)(q32;q21) in chronic lymphocytic leukemia patients: Report of two cases and a literature review

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Abstract. The chromosomal abnormality t(14;18)(q32;q21) is most commonly associated with germinal center-derived B-cell lymphomas, particularly follicular lymphoma (FL). Generally, it is considered a hallmark of FL. The t(14;18)(q32;q21) translocation is rare in chronic lymphocytic leukemia (CLL) and its prognostic significance remains unclear. In the present study, two cases of CLL with t(14;18)(q32;q21) were diagnosed using conventional cytogenetic analysis and fluorescence *in situ* hybridization. Both patients presented with leukemia and the morphological features and immunophenotypes were typical of CLL. Case 2 underwent a further lymph node biopsy, which established a diagnosis of CD5⁺ CLL/small lymphocyte lymphoma. In addition to t(14;18)(q32;q21), trisomy 12 was identified in the same clone in Case 2. Both cases exhibited immunoglobulin heavy chain variable mutations, and heavy-chain variable region gene (VH) 4-39 and VH3-62 were used in Case 1 and Case 2, respectively. In addition, direct Sanger sequencing of exons 4-9 revealed that Case 2 harbored the tumor protein p53 mutation, c.829T>G. Both cases had indications for therapy. Case 1 responded well to chlorambucil treatment, and was still alive at the last follow-up. Conversely, Case 2 exhibited aggressive disease that appeared refractory to treatment, and eventually succumbed to the disease.

Introduction

Chronic lymphocytic leukemia (CLL) is a hematological neoplasm, which is characterized by clonal proliferation and accumulation of small round B lymphocytes within the bone marrow, peripheral blood, lymph nodes and spleen (1). As the most common type of adult leukemia in western countries, the age-adjusted incidence of CLL is 4.1/100,000 in the USA (1). Annually, there are >15,000 newly diagnosed cases of CLL, and ~4,500 deaths (1). Only patients with indications for therapy should be treated. For otherwise healthy patients, immunochemotherapy consisting of rituximab, fludarabine and cyclophosphamide remains the current standard therapy (1). Conversely, for unfit patients, rituximab plus chlorambucil represents the mainstay of treatment (1). Patients with aberrations (deletions or mutations) in the tumor protein p53 (TP53) gene typically have a poor prognosis (1). The immunophenotype of neoplastic CLL cells is characterized by the coexpression of cluster of differentiation (CD)5 and CD23, weak expression of CD20, CD79b and surface immunoglobulin (Ig), as well as negative CD10 and FMC7 expression (2).

Chromosomal abnormalities are identified in ~80% of CLL patients, the most common of which are deletions in chromosomes 13q14, 11q22, 17p13 and 6q21 and trisomy 12 (3). The t(14;18)(q32;q21) translocation, which involves the immunoglobulin heavy chain (IGH) locus and the B-cell CLL/B-cell lymphoma 2 (BCL2) gene, is considered a genetic hallmark of germinal center (GC)-derived B-cell lymphomas and follicular lymphoma (FL) in particular (4-6). However, t(14;18)(q32;q21) is rare in CLL and its prognostic significance remains unclear (4).

In this report, the clinical, morphological, immunophenotypic, cytogenetic and molecular genetic findings of two cases of CLL with t(14;18)(q32;q21) are presented. Written informed consent was obtained from the patient or the patient's family for the publication of this study.

Case report

Case 1. A 46-year-old man was admitted to The Affiliated Jiangyin Hospital of Southeast University Medical College (Jiangyin, China) in August 2011 with a recurrent mild fever, which had lasted for approximately 2 years and was

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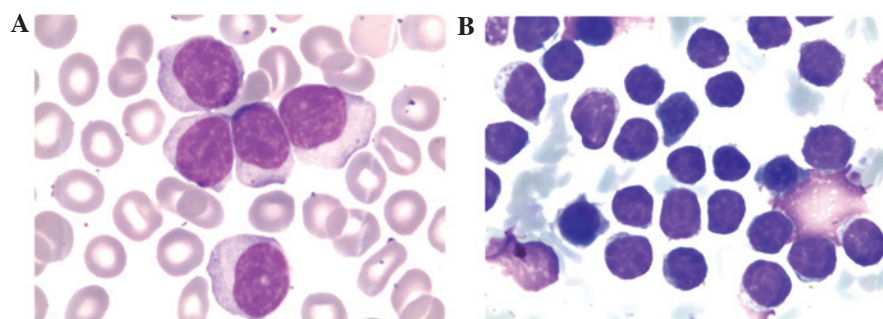


Figure 1. Case 1. (A) Peripheral blood smear revealed small mature lymphocytes (Wright-Giemsa staining; magnification, x1,000). (B) Bone marrow smear revealed numerous small mature lymphocytes without cleaved nuclei or plasmacytoid differentiation (Wright-Giemsa staining; magnification, x1,000).

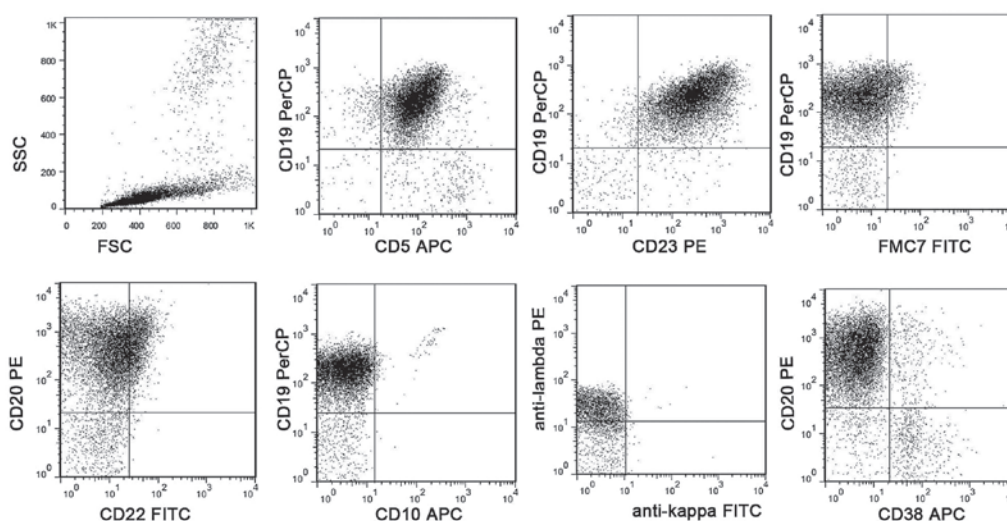


Figure 2. Case 1. Immunophenotyping by flow cytometry. Neoplastic cells were positive for CD19, CD5, CD23, with dim CD20 expression, and negative for FMC7, CD10 and CD38. CD, cluster of differentiation; APC, allophycocyanin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; FSC, forward scatter; SSC, side scatter; PerCP, Peridinin Chlorophyll Protein Complex.

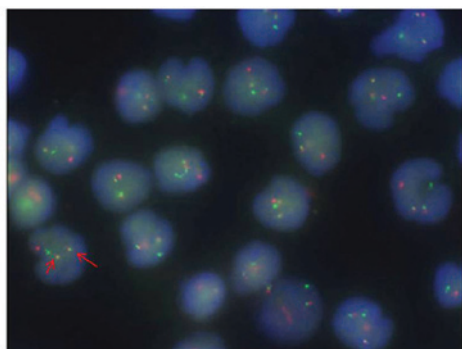


Figure 3. Case 1. Fluorescence *in situ* hybridization analysis. The neoplastic cells exhibited fusion of immunoglobulin heavy chain and B-cell lymphoma 2 (arrows), which indicated the presence of t(14;18)(q32;q21).

associated with night sweats and weight loss. Routine blood tests in September 2009 had revealed a white blood cell count (WBC) of 13,460/ μ l (normal range, 3,500-9,500/ μ l) with 69.3% lymphocytes (normal range, 20.0-50.0%); however, no further examinations were performed and no treatment was administered.

Computed tomography (CT) scans in December 2012 revealed extensive enlarged lymph nodes in the neck and

moderate hepatosplenomegaly. A complete blood examination revealed the following: WBC, 22,310/ μ l [neutrophils, 22.3% (normal range, 40.0-75.0%); lymphocytes, 74.7%]; hemoglobin, 168 g/l (normal range, 115-150 g/l); platelet count, 181,000/ μ l (normal range, 125,000-350,000/ μ l). Peripheral blood and bone marrow aspiration revealed small mature lymphocytes without indented or cleft nuclei (Fig. 1A and B). Flow cytometry (FCM) using bone marrow aspirate identified a clonal B lymphocyte population that expressed positivity for CD5, CD19, CD20, CD23, λ light chain, CD10, and CD38, dim expression of CD22 and negativity for FMC7 and κ light chain, which indicated a diagnosis of CLL (Fig. 2). Conventional cytogenetic analysis of the bone marrow aspirate revealed t(14;18)(q32;q21) in 6 of 18 metaphases analyzed. Fluorescence *in situ* hybridization (FISH) confirmed the presence of IGH-BCL2 fusion in 47% of analyzed nuclei (Fig. 3). Analysis of IGHV gene rearrangements revealed that the tumor cells exhibited a mutated IGHV gene with heavy-chain variable region gene (VH) 4-39 usage.

In April 2013, the patient experienced abdominal distention and physical examination revealed an enlarged spleen. The patient's WBC count had increased to 52,270/ μ l, with 65.0% lymphocytes. Due to persistent abdominal discomfort,

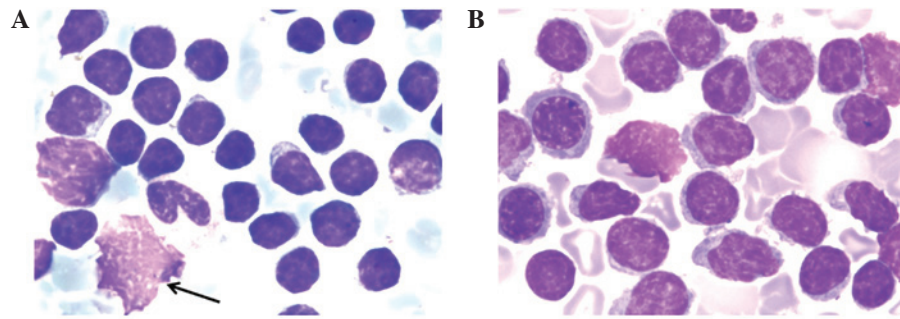


Figure 4. Case 2. (A) Peripheral blood smear revealed lymphocytosis with 16% smudge cells (arrow; Wright-Giemsa staining; magnification, x1,000). (B) Bone marrow smear revealed numerous small mature lymphocytes without cleaved nuclei or plasmacytoid differentiation (Wright-Giemsa staining; magnification, x1,000).

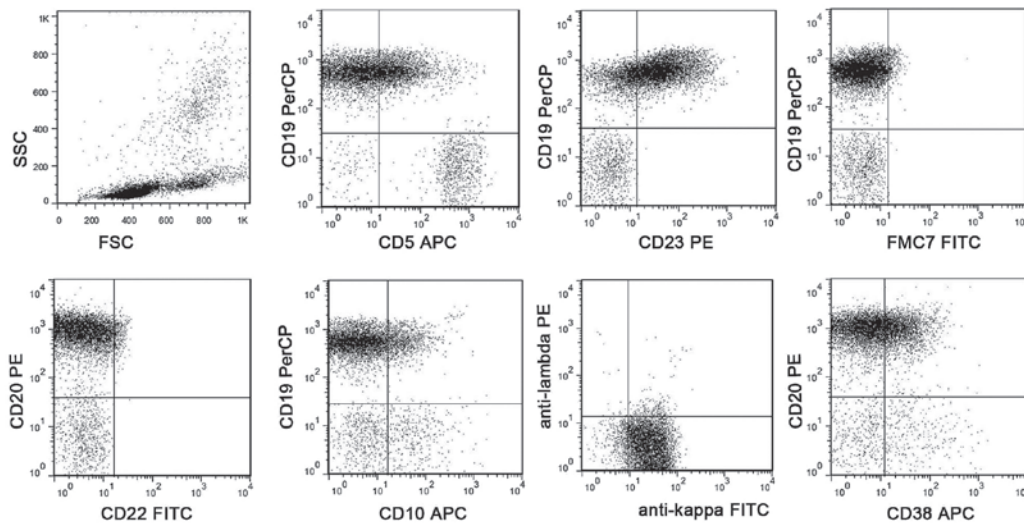


Figure 5. Case 2. Immunophenotyping by flow cytometry. Neoplastic cells were positive for CD19, CD5, CD23, with dim CD20 expression, and negative for FMC7, CD10 and CD38. CD, cluster of differentiation; APC, allophycocyanin; PE, phycoerythrin; FITC, fluorescein isothiocyanate; FSC, forward scatter; SSC, side scatter; PerCP, Peridinin Chlorophyll Protein Complex.

the patient received 12 cycles of oral chlorambucil (0.4 mg/kg body weight on days 1 and 15 of every 28-day cycle). The patient is currently in remission and undergoing follow-up.

Case 2. A 65-year-old woman presented with syncope at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China) in October 2013. Physical examination revealed enlarged cervical, axillary and inguinal lymph nodes, measuring 2-3 cm in diameter. Cranial CT scans revealed no abnormalities, however, CT scans of the chest and abdomen identified extensive enlarged bilateral axillary, mediastinal and inguinal lymph nodes. Routine blood examination revealed a WBC of 58,680 μ l with 91.3% lymphocytes, a hemoglobin level of 111 g/l and a platelet count of 110,000 μ l. Peripheral blood smear demonstrated lymphocytosis with 16% smudge cells (Fig. 4A). Bone marrow aspiration smear revealed numerous small mature lymphocytes without cleaved nuclei or plasmacytoid differentiation (Fig. 4B). FCM revealed that the neoplastic cells were positive for CD19, CD5, CD23 and CD20 with dim CD22 expression and restricted light chain expression, and negative for CD10, CD38 and FMC7 (Fig. 5). Immunophenotyping of bone marrow biopsy specimen demonstrated that

the neoplastic cells were negative for CD10 and positive for BCL2 (Fig. 6). Conventional cytogenetic study revealed trisomy 12 and t(14;18)(q32;q21) in 14 of 20 metaphases analyzed (Fig. 7). Subsequent FISH analysis confirmed these abnormalities (Fig. 8). Immunophenotyping of a cervical lymph node biopsy specimen revealed that the tumor cells were positive for CD20, PAX-5, BCL2 and CD79a, and negative for CD5, CD10, BCL6 and cyclin D1. Analysis of IGHV rearrangements demonstrated mutational IGHV status using VH3-62. Direct Sanger sequencing of exons 4-9 revealed that the patient harbored the TP53 mutation c.829T>G, without any myeloid differentiation primary response gene 88, splicing factor 3B subunit 1, NOTCH1 or BRIC3 mutations. Due to persistent night sweating that had lasted for >6 months, the patient received six cycles of bendamustine (100 mg/m²/day on days 1 and 2 of a 28-day cycle). However, an enhanced CT scan revealed that the size of the lymph nodes was increased by 180%, which indicated disease progression. The patient subsequently received three 14-day cycles of intravenous rituximab (375 mg/m² for the first cycle and 500 mg/m² for the second and third cycles). However, the patient's disease progressed rapidly and she succumbed to the disease in May 2015.

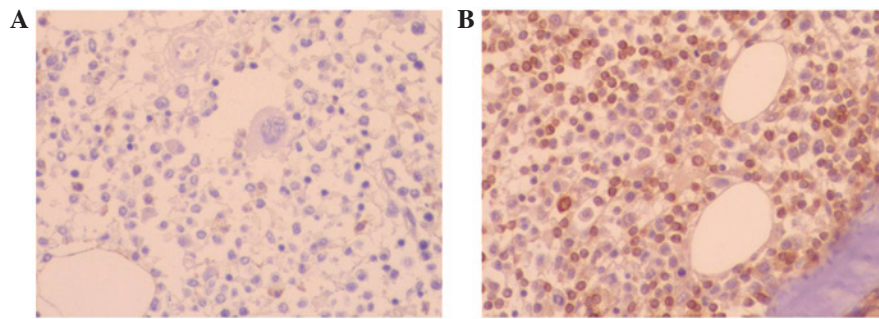


Figure 6. Case 2. Pathological examination of bone marrow specimen revealed that the tumor cells were (A) negative for cluster of differentiation 10 and (B) positive for B-cell lymphoma 2 (magnification, x400).

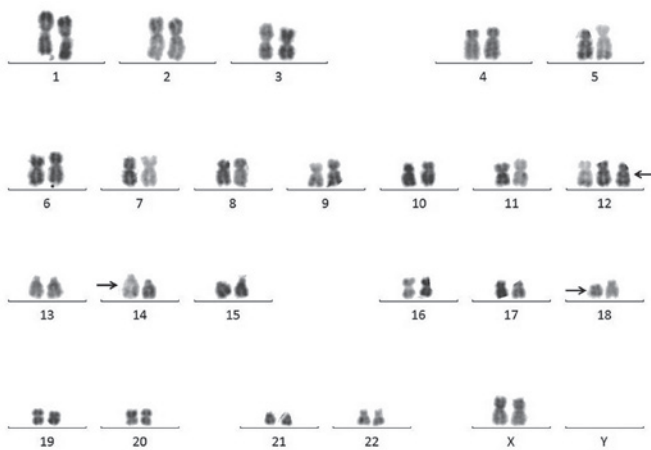


Figure 7. Case 2. Karyotype analysis revealed trisomy 12 and t(14;18)(q32;q21) in analyzed cells. Arrows indicate chromosomal abnormalities.

Discussion

The t(14;18)(q32;q21) chromosomal abnormality involves the immunoglobulin heavy chain (IGH) gene on chromosome 14q32 and the B-cell CLL/BCL2 gene on chromosome 18q21, and results in BCL2 being placed under the regulatory control of the IgH promoter leading to overexpression of the BCL2 protein (7). It is considered the genetic hallmark of FL and is identified in $\leq 90\%$ of FL cases (7). Although present in the majority of FL patients, using a standardized, highly sensitive quantitative polymerase chain reaction technique, t(14;18)(q32;q21) may be identified at low frequencies in $\leq 70\%$ healthy individuals, suggesting that BCL2 overexpression is required but not sufficient for FL development (8). It is also identified in 20-30% of diffuse large B-cell lymphoma cases that presumably originate from follicle center cells, however, it is extremely rare in CLL (4-6). Less than 2% of CLL patients harbor t(14;18)(q32;q21) (5,9-14). In the present report, two rare cases of CLL patients that exhibited the t(14;18)(q32;q21) chromosomal abnormality were presented. In these two patients, t(14;18)(q32;q21) was identified by conventional cytogenetics and FISH analysis. Case 1 exhibited an indolent clinical course, however, case 2 exhibited aggressive disease that was refractory to treatment, possibly due to TP53 mutation, which is predictive of worse outcome in CLL (15). CLL is characterized by clonal proliferation of mature B lymphocytes in the peripheral blood, bone marrow, spleen and lymph

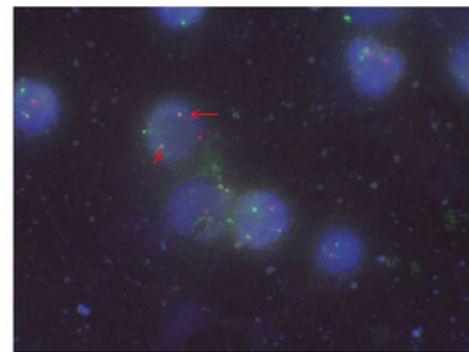


Figure 8. Case 2. Fluorescence *in situ* hybridization analysis. The neoplastic cells exhibited fusion of immunoglobulin heavy chain and B-cell lymphoma 2 (arrows), which indicated the presence of t(14;18)(q32;q21).

nodes. Diagnosis of CLL is based on the typical morphology and characteristic immunophenotype of lymphocytes. Patients with CLL that harbor t(14;18)(q32;q21) and exhibit an atypical immune phenotype may present CD5-positive FL (6,7). However, CD5-positive FL is extremely rare and <40 cases have been reported in the literature to date (16). In this study, the lymphocyte count of the two cases was $>5 \times 10^9/l$ and bone marrow examination revealed the presence of small mature lymphocytes. Both cases exhibited a CD5⁺ phenotype, which is typical of CLL. In addition, further histopathological examination of an enlarged lymph node in case 2 confirmed the diagnosis of CLL/small lymphocyte lymphoma. Furthermore, both cases were negative for CD10 expression. Based on these results, the two patients were diagnosed with CLL with t(14;18)(q32;q21). However, in cases that exhibit an atypical immune phenotype (Matutes-Catovsky score, <4) (17), if t(14;18)(q32;q21) is present, the histopathological examination of lymph nodes, spleen and bone marrow is required to exclude a diagnosis of FL (17).

Trisomy 12, which is one of the most common chromosomal abnormalities observed in CLL, is identified in 10-20% of cases by conventional cytogenetic analysis (18). The incidence of trisomy 12 in CLL with t(14;18)(q32;q21) ranges from 35-50%, which is markedly higher than that in CLL patients without t(14;18)(q32;q21) (4,6,11,13,19). Generally, CLL cases with trisomy 12 exhibit an atypical morphology and immunophenotype (20). Consistent with these findings, previous studies have reported that CLL cases with t(14;18)(q32;q21) and trisomy 12 also tend to be morphologically

and/or immunophenotypically atypical (11,21). The mechanisms underlying the frequent occurrence of trisomy 12 in CLL with t(14;18)(q32;q21) remains to be determined; however, we postulate that these two aberrations may cooperate with each other in the initiation or evolution of CLL.

IGHV somatic mutation status is one of the most important independent prognostic factors in patients with CLL (9). Genetic analysis of the IGHV somatic mutation status in CLL patients has identified two prognostic subtypes: Patients with unmutated IGHV genes exhibit a poorer prognosis than those with mutated IGHV genes, with a median survival time of 8 and 24 years, respectively (22). The most common VH subtype used is the VH3 family (40-50%), followed by VH4 family (25-33%) and VH1 family (10-17%). The VH3-21 is an independent poor prognostic factor of CLL (23). The majority of CLL cases with the t(14;18)(q32;q21) translocation (87.5-90%) harbor mutated IGHV genes (4,19). Approximately 75% of them used the VH3 family, with none of them using VH3-21, an independent predictor of poor prognosis in CLL (23). Based on these findings, the mutation rate of IGHV in CLL patients with t(14;18)(q32;q21) appears to be higher than in CLL without the chromosomal abnormality (~60%) (24). Consistent with these findings, the two patients in this report exhibited IGHV gene mutations. These findings indicate that CLL patients with t(14;18)(q32;q21) are more likely to exhibit IGHV somatic mutations and the most common VH usage is VH3 family (except VH3-21).

The t(14;18)(q32;q21) translocation is a marker of follicular center cell origin. Thus, when it occurs in the cases of CLL, it may represent differentiation toward follicular center cells (4,6). A number of previous studies have investigated the function of t(14;18)(q32;q21) in the pathogenesis of CLL (4,6,25). In two patients, the t(14;18)(q32;q21) or its variant was identified as a subclonal aberration with trisomy 12 as the primary change (4). These results indicated that in certain patients, t(14;18) may represent a secondary aberration, which may not be responsible for the onset of disease (4). Numerous CLL patients acquire novel abnormalities during the course of disease, which further supports this hypothesis (25). However, Tang *et al* (19), identified t(14;18)(q32;q21) in the stemline of 10 CLL cases and identified as the only karyotypic abnormality in 2 cases. As a result, Tang *et al* proposed that t(14;18)(q32;q21) was an early pathogenetic event in this small subset of CLL cases. Baseggio *et al* (6) revealed that the BCL6 mutation load in t(14;18)-positive CLL was lower than GC normal B-cells or GC-derived B-cell lymphoma cells. However, the involvement of t(14;18)(q32;q21) in the initiation and evolution of CLL remains controversial.

At present, the prognostic significance of t(14;18)(q32;q21) in CLL remains controversial. Certain studies have demonstrated that the CLL patients with translocations involving IGH exhibit a poorer prognosis (21,26). However, in CLL, IGH translocation partners include BCL2, BCL3, BCL11A and c-Myc, with BCL2 accounting for only a small subset of these genes (27). Nowakowski *et al* (28) observed a relatively short median progression free survival time of 20.6 months for 8 CLL patients with IGH/BCL2 fusion. Furthermore, Tang *et al* (19) revealed that t(14;18)(q32;q21) was associated with requirement for chemotherapy and possibly poorer survival (19). By contrast to these findings, Put *et al* (4)

revealed that t(14;18)(q32;q21) in CLL was not associated with an unfavorable clinical outcome in a large patient cohort with a median treatment free survival time of 48 months. In addition, all treated patients responded well to therapy (4).

In conclusion, the diagnosis of CLL is mainly based on the typical morphology and immunophenotype of neoplastic cells. The presence of t(14;18) should not be used to exclude a diagnosis of CLL. Trisomy 12 and somatically mutated IGHV genes (except VH3-21) are more common in CLL patients with t(14;18)(q32;q21) chromosomal abnormalities. Furthermore, the exact prognostic value of t(14;18) in CLL remains to be determined.

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