

Case Report

Simultaneous Occurrence of t(14;18) and t(8;22) in Burkitt's Lymphoma

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ABSTRACT

We describe a patient, diagnosed as having Burkitt's lymphoma showing plasmacytoid differentiation, with a rare karyotypic abnormality, a combination of t(14;18) and a variant translocation t(8;22). At the time of diagnosis, cytogenetic analysis revealed the presence of the two clones represented by both translocations and extra chromosomes 7,12,17 and 19, respectively.

Cytogenetic analysis after treatment at the time of relapse revealed a new clone, 48,XY,+7,t(8;22)(q24;q11),+12,t(14;18)(q32;q21) in all examined metaphases. The combination of t(14;18) and t(8;22) translocations together with numerical anomalies documented in our patient is unusual and has not been previously reported.

KEYWORDS: burkitt's lymphoma, BCL2, C-MYC

INTRODUCTION

Burkitt's lymphoma is a distinct clinicopathologic entity associated with a characteristic histologic appearance. In addition to classical Burkitt's lymphoma, two other histological variants of Burkitt's lymphoma are recognized - Burkitt's lymphoma with plasmacytoid differentiation and atypical Burkitt/Burkitt-like lymphoma^[1,2]. The constant feature of the disease is the presence of the chromosomal translocation between chromosome 8 and either chromosomes 14, 2 or 22^[3]. According to WHO classification, the evidence of the translocation represents a condition *sine qua non* for a diagnosis of all three histological variants. As a result of the translocation, the C-MYC gene sequences from 8q24 are juxtaposed with sequences from the immunoglobulin heavy chain (IgH) locus at 14q32 and, more rarely, immunoglobulin (Ig) light-chain loci at 2p12 and 22q11, respectively. While the more frequent (8;14) translocation (90% of these translocations), leads to a head-to-head fusion of C-MYC with the IgH locus, in both t(2;8) and t(8;22), the rearrangement with respectively Igk or Ig and C-MYC is head-to-tail. The common feature of the three translocations is that MYC comes to lie 5' in relation to one of the Ig gene, resulting in the abnormal regulation of the gene^[4,5].

On the other hand, the chromosomal translocation t(14;18), bringing the IgH gene sequences from 14q32 into close contact with the

BCL2 oncogene, is highly characteristic of follicular non-Hodgkin's lymphoma (NHL). As a result of the activity of the juxtaposed Ig gene enhancer sequences, this rearrangement is thought to lead to the deregulated expression of the BCL2 gene product^[5,6].

To date, simultaneous occurrence of both C-MYC and BCL2 rearrangements in one patient has been reported only sporadically and the combination of t(14;18) with one of the translocation characteristic of Burkitt's lymphoma, seems to be a rare event^[7-17].

We report here a case of one patient diagnosed as having Burkitt's lymphoma with plasmacytoid differentiation in whom cytogenetic analysis revealed the coexistence of both types of translocations at the same cell. This case may be of interest due to the (1) rare C-MYC/BCL2 association, (2) presence of the variant (8;22) translocation, (3) presence of different clones and unusual additional anomalies, and (4) due to the fact that it followed a rapidly fatal clinical course, confirming the extremely poor outcome of patients with combined occurrence of C-MYC and BCL2 rearrangements.

CASE REPORT

A 61-year-old man presented with a 1-month history of malaise, vomiting, abdominal distention and pain, fever, night sweats and weight loss. Physical examination revealed ascites and a mass in

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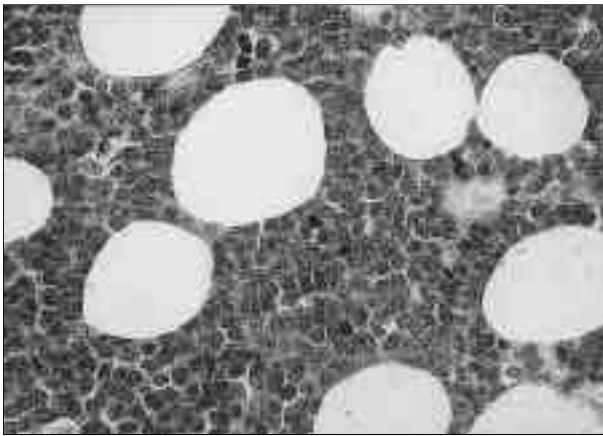


Fig. 1: Bone marrow infiltration (HE)

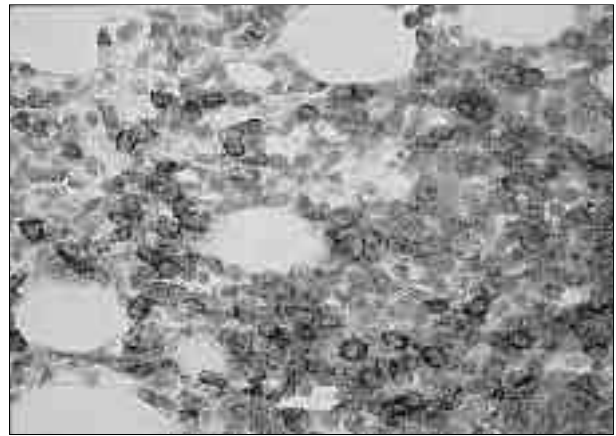


Fig. 2: Bone marrow infiltration-positivity of CD 79 alpha antigen

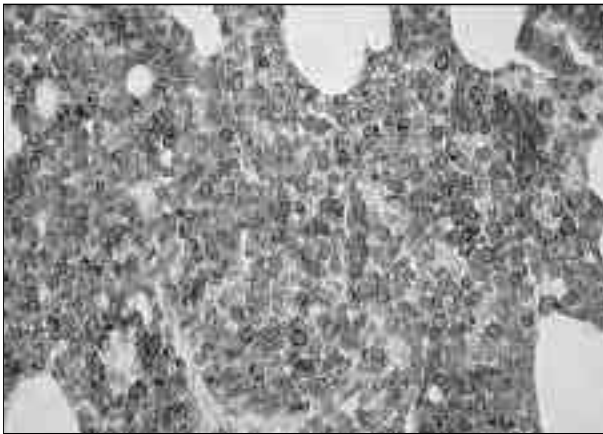


Fig. 3: Bone marrow infiltration-positivity of bcl2-protein

the upper abdomen. There was no palpable splenomegaly or peripheral lymphadenopathy. Laboratory studies showed a platelet count of 213,000/ μ L, hemoglobin level of 10.0 g/dl, hematocrit of 26.6, and WBC of 7,360/ μ L with differential of 54% segmented neutrophils segments, 10% bands, 34% lymphocytes and 2% myelocytes. Lactate dehydrogenase level was of 9540 U/L (normal range, 120 to 480 U/L). Serology was non-reactive for human immunodeficiency virus (HIV). Computed tomography revealed large masses in the upper abdomen with ascites, para-aortic lymphadenopathy and thickened wall of stomach and jejunum. Upper GI endoscopy demonstrated massive tumorous infiltration of the stomach. Bone marrow biopsy showed 70% infiltration of the marrow with abnormal lymphoid population (Fig. 1). Tissue obtained by both endoscopic and bone marrow biopsy showed a cohesive population of medium-sized cells with a diffuse monotonous pattern of infiltration and a high degree of apoptosis, as well as associated histiocytes of a "starry-sky" appearance. The tumor cells exhibited narrow rim of partially excentric basophilic cytoplasm and round nuclei with a slight degree of pleomorphism in nuclear size and

shape. The nuclei contained centrally situated one to three basophilic nucleoli. By immunohistochemical analysis, the cells expressed CD10, CD19, CD20, CD79a, bcl-2-protein (Fig. 2, 3), weak expression of monoclonal cIg with light-chain restriction, negativity of TdT and a growth fraction of nearly 100%. The diagnosis of Burkitt's lymphoma with plasmacytoid differentiation was established. The patient started chemotherapy according to the BFM protocol, with intrathecal administration of methotrexate as well as prophylactic irradiation of the brain^[18]. He achieved complete remission after two cycles of chemotherapy. He had been doing well after completion of treatment; however, six months from the initial diagnosis he was found to have relapse in the abdomen, bone marrow and central nervous system. He was subsequently treated with mitoxantrone, cytarabine, intrathecal chemotherapy and radiotherapy but ultimately died from progressive disease.

Cytogenetic analysis:

Cytogenetic preparations were made from ascites cells according to standard techniques. Cells were cultured for 24 hours without stimulation in Ischov's medium supplemented with 15% fetal serum. The analysis was carried on GTG-banding metaphases; interpretation was made according to the ISCN 1995.

Cytogenetic analysis at presentation revealed the presence of two clones. Twenty-three cells formed a stemline showing: 49,XY,+7,t(8,22)(q24;q11),+12,t(14;18)(q32;q21),and +19. Seven cells formed a sideline in the form of an extra chromosome 17 in addition to the previous chromosomal aberrations.

Six months later, a previously unidentified clone was revealed in all 50 examined metaphases: 48,XY,+7,t(8,22)(q24;q11),+12,t(14;18)(q32;q21), suggesting, the gains of chromosomes 17 and 19 are secondary chromosomal abnormalities (Fig. 4).

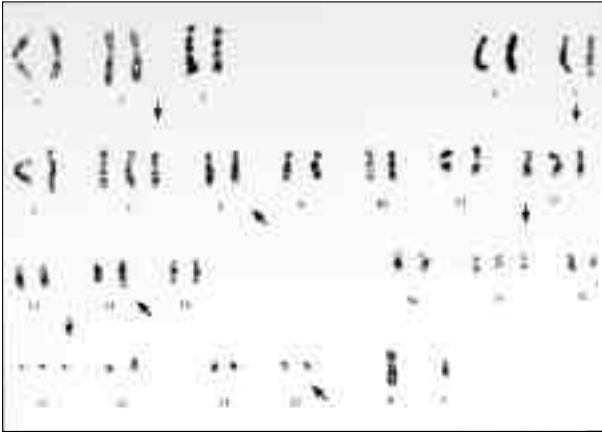


Fig. 4a: Karyotype from a patient showing the simultaneous occurrence of t(8;22)(q24;q11) and t(14;18)(q32;q21) as well as the presence of extra chromosomes 7, 12, 17 and 19 at diagnosis (a) and representative karyotype from the patient after treatment.

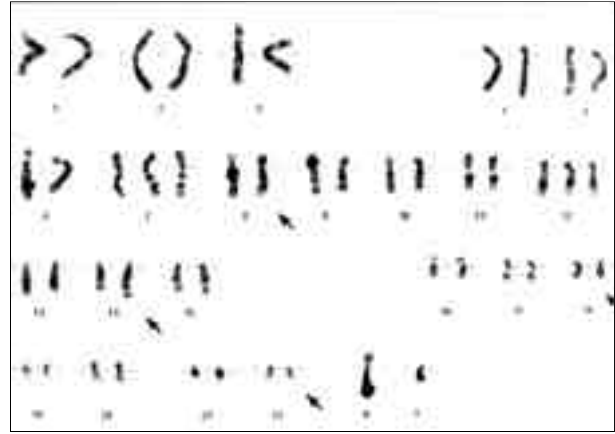


Fig. 4b: Karyotype from a patient showing the simultaneous occurrence of t(8;22)(q24;q11) and t(14;18)(q32;q21) as well as the presence of extra chromosomes 7, 12, 17 and 19 at diagnosis (b). Arrows point to abnormal karyotype from the patient after treatment.

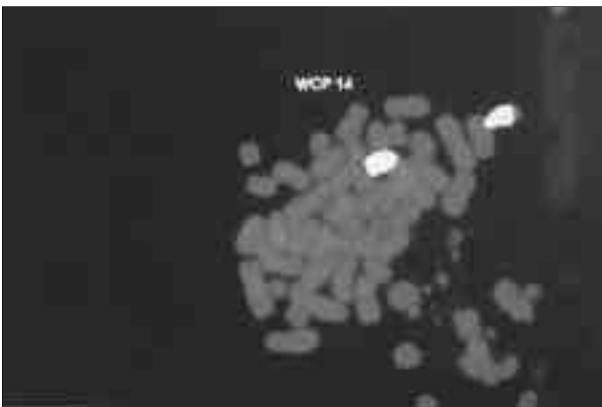


Fig. 5a



Fig. 5b

Fig. 5a-f. Whole chromosome paints of chromosomes 14 and 18 on metaphase spreads and hybridization signals with a-satellite probes showing the extra copies of chromosomes 7, 12, 17 and 19 at diagnosis.

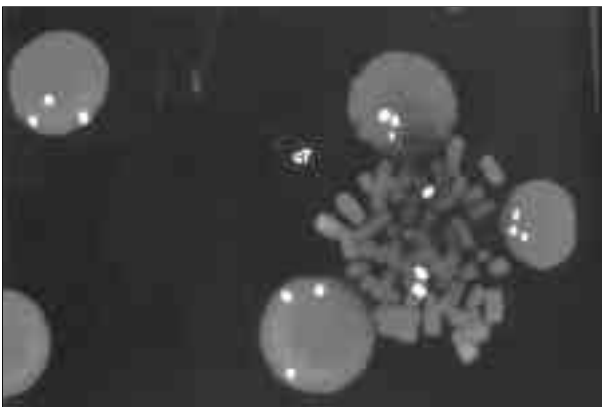


Fig. 5c

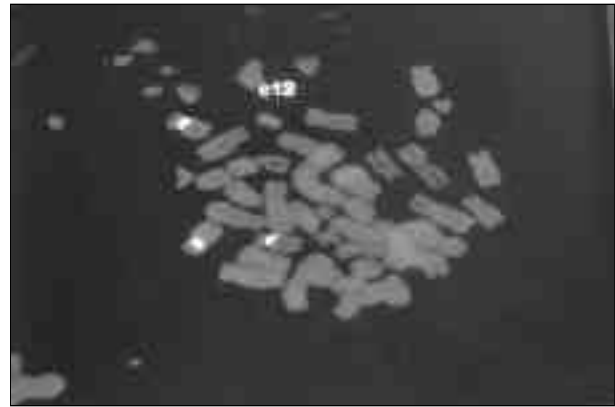


Fig. 5d

Fluorescence in situ hybridization studies:

Whole chromosome paints (WCP) and chromosome -satellite probes were hybridized on metaphase spreads and interphase nuclei according to the manufacturers' instructions (Oncor Inc., Gaithersburg, MD, USA; and Vysis Inc., Downers Grove, IL, USA). To confirm the translocations between chromosomes 14 and 8, WCPs specific for

chromosomes 14 and 18 were applied at the time of presentation. In addition, chromosome -satellite probes for the centromeric region of chromosomes 7, 12, 17 and 19 were used to identify and enumerate the respective chromosomes (Fig. 5a-f).

Molecular genetic analysis:

Molecular studies were performed using peripheral

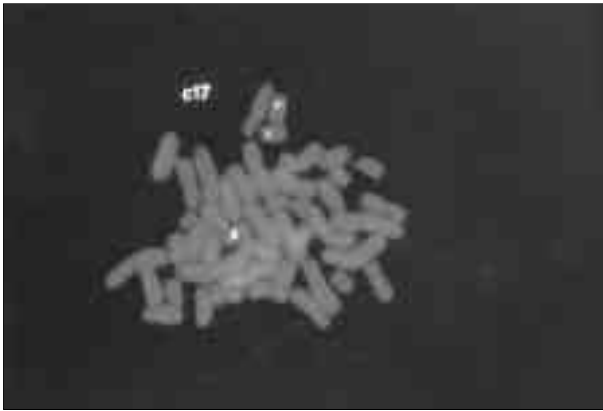


Fig. 5e

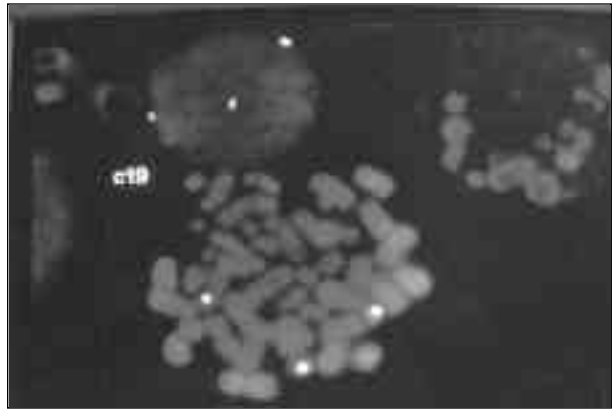


Fig. 5f

Fig. 5a-f. Whole chromosome paints of chromosomes 14 and 18 on metaphase spreads and hybridization signals with a-satellite probes showing the extra copies of chromosomes 7, 12, 17 and 19 at diagnosis.

blood mononuclear cells at diagnosis. The detection of BCL2-mbr/JH and BCL2-mcr/JH rearrangements was performed by polymerase chain reaction using the protocol of Crescenzi *et al* and Ngan *et al*, respectively^[19,20]. After subjecting the products to electrophoresis in 2% agarose gel, the product characteristic for major breakpoint region (mbr) was identified.

DISCUSSION

The chromosomal translocations t(14;18) and t(8;22) are well-known chromosomal rearrangements characteristic for follicular and Burkitt's type malignant lymphomas respectively. Follicular lymphoma is typically an indolent disease and some patients do not require any treatment for years. However, it frequently transforms into a high-grade lymphoma. The characteristic t(14;18) results in deregulate expression of the BCL2 protooncogene and increased B-cell survival. However, experiments with transgenic mice containing a BCL 2 minigene, and the observation that many normal individuals have the t(14;18) in peripheral blood lymphocytes, supports the hypothesis that the activation of BCL2 is not sufficient to render the cells malignant and additional genetic alterations are necessary to induce the malignant transformation^[21,22].

The highly characteristic anomalies in all the variants of Burkitt's tumors, the translocations t(8;14), t(2;8) and t(8;22) are variants of each other. All these translocations share a breakpoint in 8q24 (C-MYC locus) whereas in the partner chromosome the breakpoint is within one of the Ig chain loci, resulting in constitutive expression of the MYC gene. The product of the MYC gene is a transcription factor of the helix-loop-helix/leucine zipper family involved in the control of gene expression; thus its constitutive expression may result in the unrestricted proliferation of the B cell cells even in the absence of growth factors.

However, the functional basis of c-myc and bcl-2 complementation during lymphomagenesis is not well understood and is still a recurring theme of speculation. Experiments with BCL2/C-MYC hybrid transgenic mice and cell line studies revealed accelerated lymphomagenesis, thus BCL2 appears to cooperate with C-MYC in promoting cell proliferation^[23,24].

The combined occurrence of both BCL2 and C-MYC rearrangements within the same mitosis has been described only sporadically in B-cell malignancies. In this respect, some of the lymphomas spontaneously developing in BCL2 transgenic mice, in which progression from lymphoid hyperplasia led, after a long latency, to high grade lymphomas, have been shown to contain the rearranged C-MYC gene in addition to BCL2. These findings, as well as the notion that some of the patients with dual BCL2/C-MYC rearrangement had a history of documented follicular lymphoma, argue in favor of the hypothesis that the translocation t(14;18) was present in the first phase of the disease, and the acquisition of C-MYC rearrangement was accompanied by transformation. However, since our patient had no previous history of follicular lymphoma, it can be speculated that the t(14;18) is likely to be the initial event, followed by t(8;22) simultaneously or prior to transformation. In support of the hypothesis is the presence of both 7 and 12 chromosome gains in all identified clones in our patient. To the best of our knowledge, simultaneous occurrence of extra chromosomes 7 and 12 in association with t(8;22) has not been described. On the other hand, trisomies of chromosomes 7 and 12 in addition to t(14;18) are well-known secondary anomalies in follicular NHL^[25,26]. The coexistence of both t(14;18) and t(8;22) in B-cell malignancies was described in 13 patients and interestingly, in addition to dual

translocation, the gain of chromosome 7 was detected in seven cases, suggesting they could contribute to a selective growth advantage in malignant cells.

Trisomies of chromosomes 17 and 19 have rarely been detected in lymphoid malignancies. The trisomy of chromosome 17 was described in some cases of NHL in association with t(14;18). It is interesting to note that as in our patient, the extra chromosome 7 was present in half of the reported cases. The gain of chromosome 17 in association with t(14;18) and t(8;22) was reported only by Karsan *et al* in a patient with ALL, suggesting this anomaly is likely to be a secondary event. As the gain of chromosome 17, the trisomy of chromosome 19 is associated with t(14;18) in NHL, predominantly in centroblastic and large cell lymphomas.

The clinical course of our patient, as well as most of those reported in the literature, was rapidly fatal, confirming the poor outcome of patients with combined BCL2/C-MYC rearrangements. This is in agreement with the observations made in experimental systems in which the BCL2 translocation has been shown to induce prolonged cell survival by abrogating apoptosis, whereas the C-MYC translocation induces enhanced cell proliferation^[27,28]. Tumor cells with the dual translocation would therefore have a combination leading to increased cell survival associated with a bcl-2-protein over expression and proliferation resulting in rapid cell accumulation and tumor growth in a short time.

To fully understand the molecular basis of these events and to apply this knowledge for the appropriate treatment stratification will be the challenge for the future.

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