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Complex chromosomal aberrations in CLL patients — case report

Złożone zaburzenia chromosomowe u chorych z PBL-B — opis przypadku

INTRODUCTION

B-cell chronic lymphocytic leukaemia (B-CLL) is generally believed to result from the clonal expansion of mature B cells in the peripheral blood and bone marrow. The CLL cells are immunophenotypically characterized by the coexpression of CD19, CD22; low expression of CD20; absence or low expression of surface immunoglobulin (IgM or IgD), and the pan T-cell associated antigen CD5 [1]. Expression of the B-cell activation marker CD23 is also a feature of CLL lymphocytes [2, 3]. CLL often presents as an indolent disease, with some patients lingering in an early stage for prolonged periods. Other patients present with rapidly progressive disease and shorter survival [4], recognized as aggressive disease. This is associated with advancing clinical stage, lymphocyte doubling times of less than 12 months, and a high peripheral blood lymphocyte count, often accompanied by other hematologic and chromosomal abnormalities [5]. Although chemotherapy can control lymphocyte accumulation effectively in most patients, there are few complete remissions. In some cases, patients are refractory to any form of therapy.

These differences in clinical response may be related to biologic diversity in CLL, and therefore, the identification of distinct subgroups of CLL based on labolatory parameters may be relevant. The first step in this assessment is to identify correlations among characteristics such as karyotype, lymphocyte morphology, and immunophenotype.

The most frequently observed cytogenetic anomalies are trisomy 12 and the deletion or translocation of the long arm of chromosome 13, which normally includes band q14, affecting the distal portion of the retinoblastoma gene. The presence of trisomy 12 has been related to a poorer prognosis and with atypical morphologic variety of B-CLL. However the strucutral anomalies of chromosome 13q14 are not significantly correlated with a poor prognosis. Inactivation of the tumor suppressor gene p53, located in the shorted arm of chromosome 17, is a genetic alteration frequently observed in neoplasias. In B-CLL, p53 gene deletions or mutations have been detected in 10–15% of the patients.The p53 abnormalities are associated with advanced stages of the disease, transformation to Richter's syndrom, resistance to treatment, and reduced survival time.

CASE REPORT

Three B-CLL patients (three men), were hospitalized in Haematology Department, University School of Medicine in Lublin. The diagnosis of chronic lymphocytic leukaemia required a persistent lymphocytosis of greater than 5000/ml. Immunophenotypic date showed that all the cases of leukaemia were CD19+, CD5+ and CD23+. The disease was staged according to Rai classification system at the time of initiation of study. Cytogenetic studies were performed on leukemic cells using fluorescence in situ hybridization (FISH). An average of 300 cells were examined on each slide for hybridization signals.

The patients clinical and labolatory data are summarized in Table I.

Table I.

patient	sex	age	stage acc. to Rai	leukocyte count $x10^9/l$	lymphocyte count $x10^9/l$	marrow histology	TTM-score
A.F.	M	74	2	32 400	30 456	diffuse	9.5
W.J.	M	43	2	410 000	383 110	diffuse	28.6
M.J.	M	63	1	63 700	56 056	diffuse	11.4

Table II.

patients	p53 deletion (%) positive cells	trisomia 12 (%) positive cells	13q14 deletion (%) positive cells
A.F.	48.2	45.0	30.0
W.J.	38.4	0	0
M.J.	42.0	0	50.0

All the patients presented p53 deletions. The number of cells with deletions was: 48.2%; 38.4% and 42.0%. Trisomy 12 was detected in one (45.0% positive cells) and deletions at 13q14 in two cases (30.0% and 50.0% cells), cf. Table II.

All of the patients were resistant to chemotherapy treatment and died in several weeks form causes progression of CLL.

DISCUSSION

It is difficult to distinguish patients with stable or smouldering CLL from those at high risk of progressive disease [6, 7]. Risk factors for progressive disease include those which correlate with tumour load such as lymphocyte count [8], diffuse marrow histology [9], a low haemoglobin and platelet count, β2 microglobulin [10] and LDH [11], and biological parameters such as lymphocyte doubling time [12], lymphocyte morphology, karyotype and immunophenotype [13]. There is considerable interest in identifying chromosomal aberrations that could pinpoint subgroups of patients with chronic lymphocytic leukaemia who have different prognoses [14]. Conventional cytogenetic analysis has been hampered by the low mitotic activity of the leukemic cells *in vitro*. Fluorescence in situ hybridization allows the detection of chromosomal aberrations not only in dividing cells but also in interphase nuclei, an approach referred to as interphase cytogenetics. Initial studies of chronic lymphocytic leukaemia with this method demonstrated that the frequency and spectrum of chromosomal aberrations it detected differed considerably from the result obtained by conventional chromosome banding [15]. Clonal cytogenetic abnormalities can be identified in the cells from more than 50% of patients with CLL [16, 17]. Although a variety of chromosomal abnormalities have been described, trisomy 12, and structural abnormalities of the long arm of chromosome 13 at band 13q14 are most common [16, 17]. Since trisomy 12 was first discovered to be a recurring clonal abnormality in CLL, many but not all studies have suggested that trisomy 12 was associated with rapid disease progression, a need for early treatment and poor survival [18, 19, 20]. In contrast, the survival of patients with deletions or translocations of chromosome 13q14, which is the other common cytogentic abnormality in CLL, was no different from patients with a normal karyotype. More recently some researchers have drawn attention to the association between trisomy 12 and atypical lymphocyte morphology [21, 22, 23]. Mutations in p53 have been associated with disease progression and decreased survival in many human malignancies, including chronic myelogenous leukaemia [24], multiple myeloma [25], breast carcinoma [26], gastric carcinoma [27], colon adenocarcinoma [28], and lung carcionoma [29]. Despite their prevalence, p53 mutations have not been associated convincingly with prognosis for most patients with these tumors. Several investigators have presented evidence favoring a prognostic role for p53 mutations in patients with CLL. One study reperted significantly lower rates of remission and survival in patients with aggressive CLL tumors carrying p53 mutations [30], and another report associated p53 mutations with drug resistance and poor clinical outcomes [31].

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STRESZCZENIE

Przewlekła białaczka limfocytowa B-komórkowa (PBL-B) należy do najczęściej występujących białaczek w Europie Zachodniej. Charakteryzuje ją klonalna ekspansja dojrzałych limfocytów B o typowym fenotypie immunologicznym, a także bardzo różnorodny przebieg kliniczny. Około 1/3 chorych nigdy nie wymaga leczenia, u części początkowo „tląca" się choroba z

czasem ulega progresji, natomiast w pozostałej grupie chorych od samego początku przybiera agresywną postać, wymagającą natychmiastowej terapii. U ok. 50% chorych z PBL-B stwierdza się zaburzenia genetyczne. Do najczęściej spotykanych należy trisomia 12, której obecność wiąże się ze złym rokowaniem, a także występowaniem atypowych wariantów morfologicznych PBL-B. Natomiast zaburzenia strukturalne chromosomu 13q14 nie mają w chwili obecnej ściśle określonego znaczenia klinicznego. Delecje/mutacje TP53, genu zlokalizowanego na chromosomie 17, wykrywane są w 10–15% przypadków chorych z PBL-B. Ich obecność wiąże się zazwyczaj z zaawansowanym stadium klinicznym, transformacją w zespół Richtera, opornością na chemioterapię oraz krótkim czasem przeżycia. Prezentujemy przypadek trzech chorych z PBL-B, u których stwierdzono w chwili rozpoznania wysoki odsetek komórek z delecją p53, trisomią 12 oraz delecją 13q14. Wszyscy opisywani chorzy wykazali przy tym oporność na stosowane leczenie i zmarli w czasie kilku tygodni z przyczyn związanych z białaczką.