Cytogenetic Study of Bone Marrow and Blood Cells in Patients with Non-Hodgkin Lymphoma before and after Antitumor Therapy

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Non-clonal and clonal abnormal cells (hypodiploid, polyploid, and with chromosome aberrations) were detected by the G-method in the bone marrow and blood of the majority of patients with non-Hodgkin lymphomas before therapy. The manifestation of these abnormalities changed individually after treatment. The most incident were clones -22, del(1)(q42-43), del(2)(q-33-35), del(3)(q27), 4q–, and del(6q).

Key Words: *non-Hodgkin lymphomas; karyotype disorders; clonal abnormalities*

Treatment of non-Hodgkin lymphomas (NHL) sometimes leads to the development of the resistance to antitumor therapy (AT); transformation of lymphomas of low malignancy into highly malignant lymphomas, and to the development of secondary tumors with time. These changes are probably associated with genome instability of lymphoid cell persisting even after effective AT [8-11].

The majority of cytogenetic studies of NHL are focused on the analysis of karyotype of lymph node cells. The data on the genome of bone marrow and blood cells are scanty, though blood lymphocytes are considered to be the most sensitive indicators of physical and chemical mutagenesis and retain karyotype disorders during many years [11]. Since the majority of antitumor drugs directly or indirectly impair DNA synthesis and replication [1,2], the increase in the incidence of genome and chromosome aberrations in blood lymphocytes after AT can indicate patient hypersensitivity to the damaging effect of AT and/or its intensity. Evaluation of these parameters is essential for optimizing AT protocols and reducing the therapeutic risk [2,7,11].

The aim of this study was to determine cytogenetic parameters characterizing the bone marrow and blood cell genome before and after therapy.

MATERIALS AND METHODS

Cytogenetic study of bone marrow and blood cells was carried out in 42 patients with NHL (23 men and 19 women aged 16-74 years) before AT and 19 patients (11 men and 8 women aged 29-61 years) during various periods (3 to 21 months) after single course of AT. Standard chemotherapy protocols were used. Four patients received radiotherapy in addition to chemotherapy. The diagnosis of NHL was verified by traditional morphological and immunohistochemical analysis of the tumor and immunophenotyping of blood lymphocytes. By the present time no strict parallelism between the morphology and cytogenetics of NHL subtypes was detected [5,8], and therefore we analyzed all data together.

Bone marrow cells were cultured for 24 h, blood cells for 72 h with routine phytohemagglutinin stimulation [10,11].

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Chromosome preparations were treated by the G-method and analyzed using a Cytoscan PC system (Applied Imaging). The percentage of hypodiploid cells, cells with chromosome aberrations, presence of a polydiploid cell strain, and clonal disorders of the karyotype were analyzed. Dicenrtic chromosomes were excluded from the analysis of chromosome aberrations, because it is known that cells with these aberrations are eliminated with time and very rarely form clones. These cells were not described as clonal cells in NHL. Twenty to fifty metaphases were analyzed.

RESULTS

Genome instability of bone marrow and blood cells was detected in all patients before AT; it manifested by genome and chromosome abnormalities of the karyotype.

Genome abnormalities manifested in aneuploidy and presence of polyploid cells. Aneuploidy was detected in bone marrow cells of 96.0±3.9% patients and in blood cells of 85.3±6.1% patients. The percentage of patients with hypodiploid cells was the highest: 100% in the bone marrow and 94.1± 4.0% in the blood. The content of hypodiploid cells in the bone marrow varied from 6.2±4.3 to 46.7± 12.9%, in the blood from 0.0 to 46.7±12.9%, the mean values being 24.8±2.4 and 21.4±2.4%, respectively. The content of hypodiploid cells in the blood surpassed the permissible level (5.2%) in the majority of patients (85.3±6.1%, data for the bone marrow were not obtained). Polyploid cells with more than 50 chromosomes were present in the bone marrow of 76.0±8.5% patients and in the blood of 44.1±8.5% patients.

Chromosome aberrations were detected in bone marrow cells of 88.0±6.5% patients and in blood cells of 94.1±4.0% patients. The percentage of cells with chromosome aberrations in the bone marrow varied from 0 to 64.0±10.5%, the mean level being 19.9±3.4%; the values for the blood were 0 to 88.9±7.4 and 23.2±4.0%, respectively. The content of cells with chromosome aberrations in blood cells surpassing the permissible level was 6.4% in the majority of patients (85.3±6.1%), with consideration for the possible impact of the culturing procedure [5]. The spectrum of chromosome aberrations of bone marrow and blood cells included predominantly deletions (in 78.0±6.5% patients), complete loss of p-arms (58.5±7.7%) and q-arms (56.1±7.7%), and translocations (39.0±7.6%). Other types of chromosome aberrations were rare (in 21.9±6.4% patients).

Clonal disturbances of the karyotype were present in the bone marrow and/or blood cells of 63.4± 7.5% patients. The most incident was the loss of chromosome 18 (in 17.1±5.9% patients) and del(6q) (in 19.5±69.2% patients). These disorders are associated with lymphoproliferative diseases [7]. Other clonal abnormalities of the karyotype repeating in different patients were -17, -20, -21, -22, del(1) (42-43), del(2)(q 35), del(3)(q 27), 4q$^-$, del(4)(31-32), 5q$^-$, 8q$^-$. Two or more clones were detected in 41.5±7.7% patients. Clones associated with myelodysplasia and/or acute myeloid leukemia were detected in 31.6±7.3% patients, in addition to the clones associated with lymphoproliferative diseases.

All patients examined before AT were divided into 2 groups (with histologically detected and undetected bone marrow involvement) for evaluating the differences by the studied cytogenetic parameters between these two groups. However, comparison showed no essential differences (Table 1). Moreover, clonal disorders of the karyotype were detected in patients without bone marrow involvement, and 36.4±7.0% patients had clonal disorders characteristic of myelodysplasia and/or acute myeloid leukemia. Presumably, genome instability in bone marrow cells detected by cytogenetic study reflects nonspecific high mutability of hemopoietic cells, caused by physical, chemical, or hereditary factors.

Repeated testing before the second course of AT showed that the content of hypodiploid cells

TABLE 1. Cytogenetic Parameters of NHL Patients with bone marrow (BM) Involvement (*M*±*m*)

Parameter	Patients with histological signs of BM involvement		Patients without histological signs of BM involvement	
	BM	blood	BM	blood
% of hypoploid cells	25.3±8.6	11.2±5.7	24.7±2.5	23.2±2.7
% of patients with polyploid cells	40.0±21.9	20.0±17.9	85.0±8.0	44.8±9.2
% of cells with chromosome aberrations of karyotype	14.0±6.7	21.0±15.4	20.9±4.1	23.5±4.1
% of patients with clonal disorders of karyotype	40.0±21.9	40.0±21.9	45.0±11.1	69.0±8.9

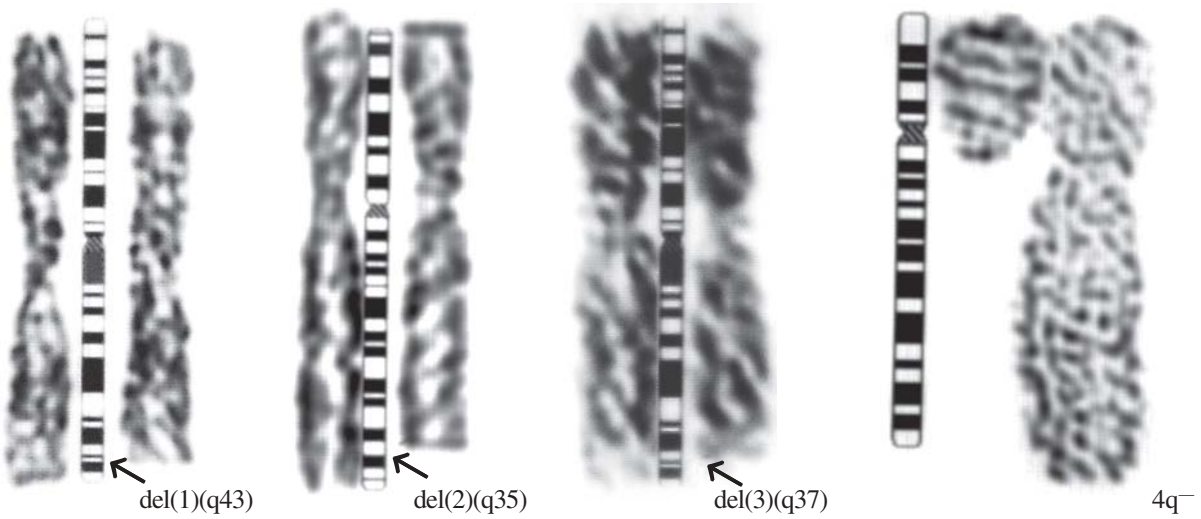

Fig. 1. Clonal chromosome aberrations in NHL patients after AT.

was retained at the same level or decreased in the majority of patients (in 85.7±9.3% in the bone marrow and in 66.7±12.2% in the blood), and in some cases these cells were not detected. The number of patients with chromosome aberrations remained unchanged or increased (23.1±11.7 in the bone marrow and 26.7±11.1% in the blood). No chromosome aberrations were detected in the bone marrow and/or blood cells of some patients, but the number of patients with translocations in bone marrow and/or blood cells increased negligibly: from 35.3±11.6% before AT to 52.9±12.1% in repeated testing. The number of patients with polyploid cells was high: 87.5±9.3% with these cells in the bone marrow and 57.1±13.2% in the blood. Polydiploid cells were not only retained in the bone marrow and/or blood of the greater part of patients, in whom they were found before AT, but emerged in other patients, in whom they were previously not detected. However, polyploid cells were not detected by repeated testing in some patients (in 14.3±9.3% in the bone marrow and in 21.4±11.0% in the blood), though they were present before AT.

Clonal disorders of the karyotype were detected in the bone marrow and/or blood in 58.8± 11.9% patients. Alteration of clonal disorders was observed (repeated testing revealed clonal disorders differing from those present before AT) and emergence of clonal disorders in the patients in whom they were not detected before AT. Only two patients had no clonal disorders before AT and these disorders were not detected at repeated testing.

Repeated testing detected clonal disorders of the karyotype, which were occasional in different patients before AT (-22, del(1)(q42-43), del(2)(q33-35), del(3)(q27), 4q⁻, del(6q); Fig. 1); similarly as before AT, del(6q) was the most incident (23.5±10.3% of patients). Presumably, these karyotype disorders are responsible for NHL development though a relationship with lymphoproliferative diseases was detected only for -22 and del(6q) aberrations [7]. Clonal disorders -5,5q⁻ associated with myelodysplasia and/or acute myeloid leukemia [7] and absent before AT were detected in 4 patients.

The detected effects did not depend on radiotherapy or its absence. The number of patients with dicentric chromosomes in the bone marrow and/or blood cells (these chromosomes are considered as markers of radiation exposure) before AT was 29.4± 11.0% and during repeated testing this parameter increased to 64.4±11.6%. Cells with dicentric chromosomes were found in patients receiving radiotherapy or chemotherapy alone.

Hence, changes in the cytogenetic picture in patients after a course of treatment corresponded to the known variants: persistence of nonclonal karyotype disorders, replacement of clonal disorders by new ones not observed before AT, and appearance of clonal disorders (including second tumor markers) which were absent before AT. However, the reaction to the course of therapy is individual in each patient. In some cases it manifests in a reduction of manifestation of some cytogenetic parameters and/or disappearance of clonal disorders, which presumably reflects the positive effect of AT. In other cases, a negative reaction was observed, presumably indicating the persistence or progress of the disease, particularly when clonal disorders characteristic of myelodysplasia and/or acute myeloid leukemia are detected, which should be taken into consideration when planning further therapeutic courses.

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