Study of Nonrandom Chromosome Aberrations in Bone Marrow and Blood Cells of Patients with Non-Hodgkin's Lymphomas

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 5, pp. 555-558, May, 2010
Original article submitted January 21, 2009

Complete karyotyping of G-stained chromosomes of bone marrow and blood cells was carried out in patients with non-Hodgkin's lymphomas before and after antitumor therapy, in remissions and relapses. Clones with 1q41-43, 2q35, and 6q24-27 deletions were most incident. Their incidence in patients increased after antitumor therapy.

Key Words: *non-Hodgkin's lymphomas; clonal chromosome aberrations*

Non-random chromosome aberrations (NRCA) are detected in patients with non-Hodgkin's lymphomas (NHL) before and after antitumor therapy (AT) [1-5,7,9,11]. It is assumed that these aberrations are a mechanism of NHL induction and progress and of formation of AT resistance. Studies of lymph node cells showed that the incidence of NRCA is higher in progressing tumors [7,9,11]. The risk of transformation of follicular NHL into diffuse large-cell NHL was maximum in patients with clonal deletions in 6q23-26 and 17p segments [9]. The relationship between 17p restructuring and NHL transformation and progress were reported [4,5]. Other authors [9,11] showed that the presence of breaks in 1p21-22, 1q21-22, 6q23-26, and 17p segments in the NHL lymph node cells was associated with a significantly worse survival of patients. According to some authors [7,11], NRCA with breaks in 1q21-22 and 6q21-25 segments were detected in NHL patients before and after AT during the early and advanced stages of the disease.

We tried to detect the most incident NRCA in bone marrow and blood cells of NHL patients before and after AT, in remissions and relapses.

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MATERIALS AND METHODS

Complete karyotyping of bone marrow and blood cells of patients with different morphological types of NHL was carried out using Cytoscan PC system (Appllied Imaging). The group included patients with different NHL morphotypes: 58 (32 men and 26 women, 16-74 years) before AT and 31 (14 men and 17 women, 29-61 years) after AT. Antitumor therapy was carried out according to the standard protocols of chemo- and radiotherapy. The status of 20 patients corresponded to partial or complete remission according to the international criteria. Five patients had 1-2 relapses, 6 had relapses with active progress of the tumor. Three to five checkups were carried out in 11 patients with lasting remissions over the periods from 3-5 months to 4-7 years after AT. Bone marrow puncture specimens (1 ml) were collected from the sternum, added to 8 ml RPMI-140 with 1 ml FCS, 0.1 ml glutamine, and 0.09 ml heparin and incubated (2 h) in a thermostat (37°C). The suspension was then divided into 2 tubes. Colchicine (0.02 μg/ml; Sigma) was added to each tube and culturing was continued for 21 h. Venous blood (1 ml) was cultured at 37°C (72 h) in nutrient medium of the following composition: 6.16 ml RPMI-140, 1.6 ml FCS, 0.08 ml L-glutamine, 0.08 ml antibiotics (penicillin and streptomycin), and 0.015 ml PHA-P. Two

0007-4888/10/1495-0621

hours before fixation, colchicine was added to a final concentration of 0.5 μg/ml. All reagents were from PanEko. Bone marrow and blood cell fixation and making of chromosome preparations were carried out by standard methods with hypotonic KCl (0.75 M) and fixative (methanol and glacial acetic acid, 3:1).

All detectable chromosome aberrations were recorded in G-stained karyotypes and NRCA, *i.e.* clonal abberations occurring at least twice in the studied cell population, were detected. Depending on the quality of preparations, 20-50 metaphases per sample were analyzed.

The results were statistically processed using Student's *t* test.

RESULTS

Before AT, NRCA were present in bone marrow and/or blood cells of 36.2±6.3% patients. Restructuring with the formation of clones was most often found in the terminal segments of q-arms of chromosomes 1, 2, and 6 (in 6.9±3.3, 10.3±3.9, and 13.7±4.5% patients, respectively) and in the pericentromer segments of p- and q-arms chromosomes 4 and 5 (in 10.3±4.0 and 8.6±3.7% patients, respectively; Fig. 1). Clones with deletions in segments 2q35 (in 10.3±3.9% patients) and 6q25 (in 13.7±4.5% patients) were the most incident. One and the same patient could have clones with deletions in different chromosomes and/or different segments of the same chromosome. These multiple clones (2 and more) were found in 17.2±4.9% patients.

The percentage of patients with NRCA increased significantly (70.9±8.2%; p=0.001) after AT. Similarly as before AT, the most damaged (with the formation of clonal disorders) after AT were the q-arm terminal segments of chromosomes 1, 2, 6 (22.5±7.5, 19.3±7.0, and 64.5±8.6% patients, respectively). Clonal deletions in the pericentromer segments of chromosome 4 p- and q-arms, chromosome 5 q-arm, and chromosome 18 p-arm were found (in 12.9±4.4, 9.7±3.9, and 9.7±3.9% patients, respectively). The most incident were clones with deletions in segments 1q41-43, 2q35, and 6q24-27, the most incident of these being the clone with del(6)(q25) (Fig. 2) detected in a greater percentage of patients than before AT (62.4±8.6%, p=0.001). Clonal deletions with breaks in other 6q segments were concomitant with del(6)(q25) and were much more rare. Similarly as before AT, there were multiple clones in

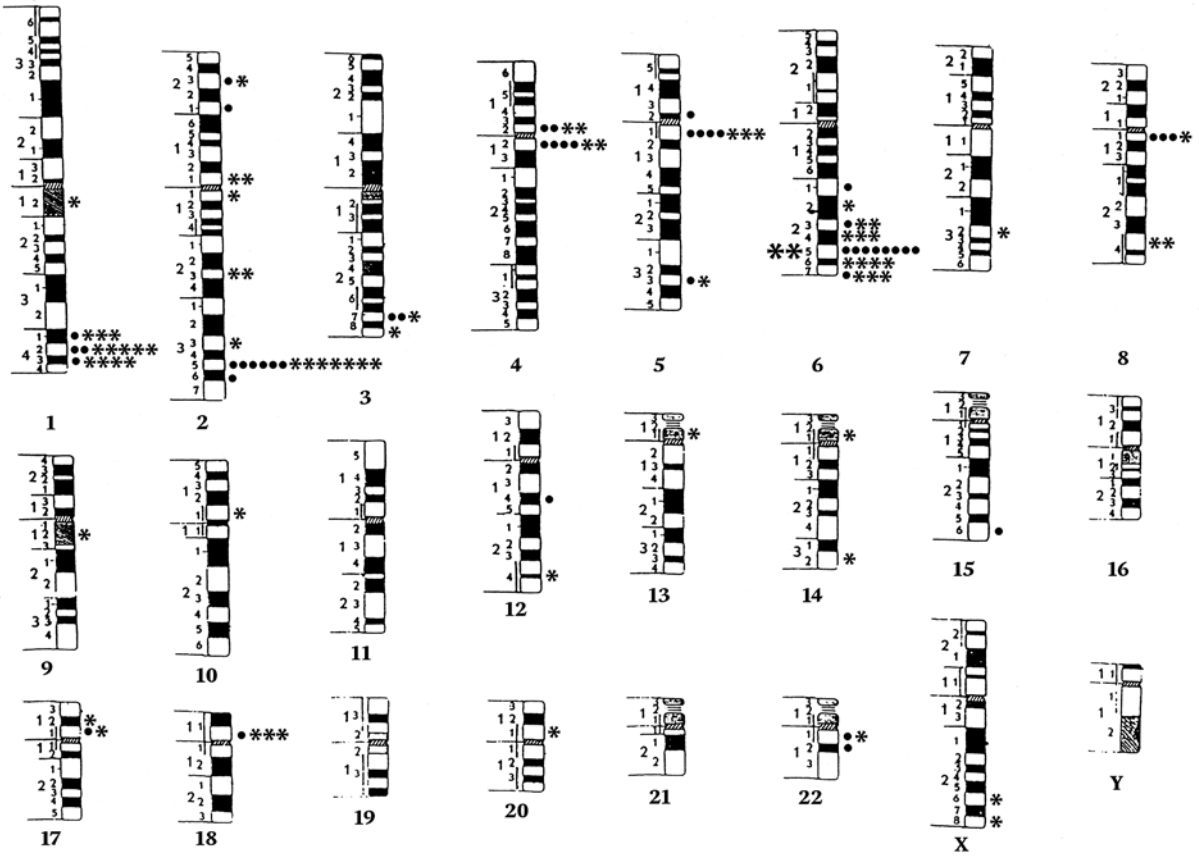

Fig. 1. Distribution of regular points of chromosome breaks in bone marrow and/or blood cells of NHL patients before (•) and after (*) AT. •*: 1 case; *: 10 cases.

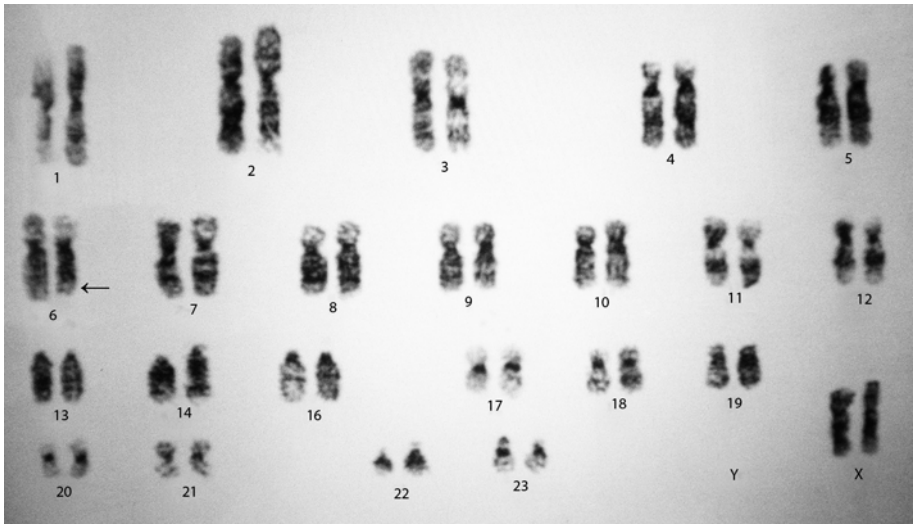

Fig. 2. Blood lymphocyte karyotype in a female patient with diffuse large-cell lymphoma (2.5 months after AT). NRCA del(6)(q25).

a high percentage of patients (29.0±8.1%) (though the presence of these clones was negligible).

In patients with remissions, NRCA were detected during the early and delayed periods after AT. Deletions in segments 1q41-43, 2q35, and 6q23-27 most often formed clones (Fig. 3) [1]. Eleven patients had multiple clones. Repeated checkups throughout long remissions detected most regularly the del(6)(q25) clone. In two patients it was detected during the early remission (5-6 months), and 1-2 years after AT it was detected in all patients repeatedly at every checkup.

In only 1 patient with relapse no NRCA were detected. The most incident clones were del(2)(q35) and del(6)(q25), and they could be present in the same patient. The 6q25 clone deletion was found in the greatest number of patients (9) irrespective of the duration of relapse after AT. It was found in patients with multiple clones and was the only in patients with a solitary clone.

In our study, the most incident disorders in bone marrow and blood cell karyotype before and after AT were deletions in 1q41-43, 2q35, and 6q23-27 segments. The presence of clones with these aberrations in some patients before AT can indicate that they are not secondary (caused by AT), while their low incidence before AT can be attributed to small size (insufficient for detection by the standard cytogenetic methods) of these clones in the bone marrow and blood cell populations. Polymorphism of one of the genes of folate metabolism system (*MTR* gene in 1q43 segment) is linked with the risk of NHL [9,10]. The same was detected for one of oxidative stress genes (*SOD2* in 6q25.3 segment) [11]. Presumably, the detected increase in the incidence of these clones in patients after AT is a result of dissemination of the genetically-determined process. The presence of these clones did not depend on the morphological type of NHL. Further studies of NRCA in NHL, particularly in patients after AT during different periods of remission and in repeating relapses, can promote the detection of the mechanisms of NHL development and help to evaluate the prognostic significance of these disorders.

The authors are grateful to E. I. Maryina, laboratory research assistant.

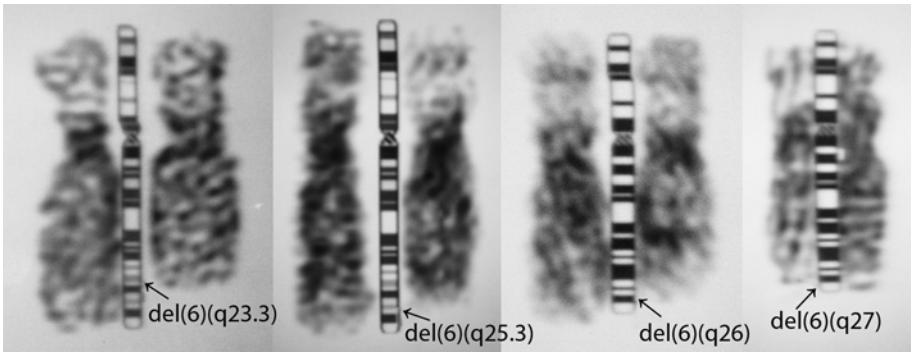

Fig. 3. Clone deletions with breaks in different segments of 6q in blood cells of NHL patients during remission.

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