THREE CASES OF ACUTE MYELOID LEUKEMIA AND DELETION OF THE LONG ARM OF CHROMOSOME 9

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Summary. Deletion of the long arm of chromosome 9 (del 9q) is a recurrent cytogenetic abnormality in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). We describe the first three de novo AML cases with del(9q) in our country, found as the sole karyotypic anomaly. Karyotyping indicated two areas of deletion – in the regions 9q21-22 and 9q13-34. There was found the association of del(9q) with characteristic phenotypic abnormalities: blasts with or without cytoplasmic granulations, dysplastic changes in granulocyte lineage, giant myeloperoxidase positive cytoplasmic granulations (pseudo-Chediak-Higashi type) and CD7(+) and CD34(+)/CD34(-) antigen expressions. In one of the patients there was also an association with dysplastic changes in erythroid, granulocytic and megakaryocytic lineages. It is likely that del(9q) is a clonal event in the pre-leukemic stage of the disease, probably in unrecognized MDS. Deletion of tumor-suppressor genes in 9q chromosome regions may relate with leukemogenesis in AML.

Key words: *acute myeloid leukemia, 9q abnormalities, cytogenetics of acute leukemia*

INTRODUCTION

Deletion of the long arm of chromosome 9 (del9q) is a recurrent cytogenetic abnormality in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [1, 4]. In contrast to many cytogenetic abnormalities, little is currently known about this chromosomal deletion. Del (9q) was reported predominantly as an additional cytogenetic aberration, especially together with t(8;21) [1, 6]. The breakpoint region, which is deleted, can differ in size, such being the case with 5q-, 7q- and 20q- deletions [9, 11]. Recent evidence revealed

that del(9q) is another cytogenetic variant of AML and is associated with specific clinical and laboratory findings [9]. According to WHO classification of myeloid neoplasms, del(9q) has diagnostic significance and is recurrent unbalanced chromosomal change in AML with myelodysplasia-related changes [10].

The aim of the study was to evaluate the clinical, morphological, immunological and cytogenetic features of AML with 9q deletions.

MATHERIALS AND METHODS

Patients: Three patients with diagnostic del(9q) abnormalities were reported. Patients did not have history of previous chemotherapy or radiotherapy. The diagnosis of AML was based on morphologic and cytochemical studies of peripheral blood smears and bone marrow aspirate obtained before therapy was initiated.

Cytological characterization: Bone marrow aspirates were stained with May–Grunwald–Giemsa. Cytological features initially assessed were: the degree of myeloid/monocytoid differentiation; nuclear appearances; cytoplasm appearances; myeloid dysplasia; erythroid dysplasia; megakaryocytic appearances.

Cytochemical methods: We used cytochemical reactions for myeloperoxidase (according to G. Graham – W. Knoll) and for α-NAE (according to H. Loffler).

Immunophenotyping: Leukemic cells were analyzed from fresh bone marrow or peripheral blood samples collected in EDTA-containing tubes. Surface, cytoplasmic and nuclear antigens were detected by a standard two-color direct immunofluorescent assay, with the use of a broad panel of lymphoid and myeloid-associated, commercially available monoclonal antibodies (MoAbs). These included: MoAb to the pan-leukocyte antigen CD45; myeloid-associated MoAbs (MPO, cyPOX, CD11b, CD11c, CD13, CD14, CD15, CD33, CD64, CD117); B-cell-associated MoAbs (CD19, CD20, CD21, CD22, CD24, surface immunoglobulins (sIg), cytoplasmic – cyCD79a, cyIgM); T-cell-associated MoAbs (CD1a, CD2, CD3, CD4, CD7, CD8); platelet-associated MoAbs (CD42a, CD9); erythrocyte- associated MoAbs (Glycophorin A, CD71) and non-lineage specific MoAbs (CD10, CD34, CD38, TdT, HLA-DR). Fluorescent labeling was evaluated by flow cytometer using a FACSort (Becton Dickinson Immunocytometry Systems, Mountain View, CA).

Conventional cytogenetics: For the purpose of conventional cytogenetics we used bone marrow cells, prepared by direct method and after 24 hours of cell cultivation. The chromosomes were stained by G-banding method and karyotypes were determined according to ISHC (International System for Human Cytogenetic) nomenclature [5].

RESULTS

Patient characteristics

The clinical and biological characteristics of patients are summarized in Table 1.

Table 1. Clinical and biological characteristics of patients with AML and del(9q)

Parameters	Patient № 1 Male/32yr	Patient № 2 Male/47yr	Patient №3 Female/44yr
Fatigue	significant	significant	significant
Weight loss	significant	significant	significant
Fever	significant	significant	slight
Unusual bleeding	gums bleeding/ skin rush	gums bleeding/ skin rush	no
Hepatomegaly/splenomegaly	slight	slight	no
Lymphadenopathy	no	no	no
CNS involvement	no	no	no

CNS-Central nervous system

The laboratory characteristics of the patients are summarized in Table 2.

Table 2. Laboratory characteristics of patients with AML and del (9q)

Clinical symptoms	Patient № 1	Patient № 2	Patient № 3
Hemoglobin (g/l)	69	80	70
WBC ($x10^9$/l)	68	41	9.5
PLT ($x10^9$/l)	29	86	157
Immature granulocytes (PB)	(-)	(+)	(-)
PB blasts (%)	92	86	(-)
BM myelo/monoblasts (%)	22/48	20/68	16/5
Myeloperoxydase staining	(+++)	(+++)	Not performed

WBC – white blood cells; PLT – platelets; BM – bone marrow; PB – peripheral blood

Cytological characteristics: According to FAB classification all cases were determined as AML type M4 (acute myelo-monocytic leukemia). Bone marrow myeloblasts of patient № 1 were hypogranular (Fig. 1A). Patients № 2 and № 3 had prominent cytoplasmic granules (Fig. 1B and 1C). Bone marrow dysplasia of granulocyte, erythrocyte and megakaryocyte precursors was established in patient N1 (Fig 2). There were erythroblasts with megaloblastoid changes, irregular nucleus, multinucleated form, internuclear bridges and basophilic cytoplasm. The granulocyte cell line was characterized with cytoplasmic hypogranulation, basophilic staining of peripheral cell cytoplasm, hypersegmentation of the nucleus, ring forms of nuclei and chromatin clumping. Dysplastic megakaryocytes were small, with scant cytoplasm and hypo-lobulated nuclei.

Positive results of cytochemical reactions for myeloperoxidase and α-NAE were established in patients № 1 and № 2 with giant myeloperoxidase positive granules (pseudo-Chediak-Higashi granules) (Fig. 3).

Immunophenotyping: CD34 and CD7 were expressed in patient № 1. Patient № 2 showed CD34 and CD7 negative blasts (Fig. 4 and 5).

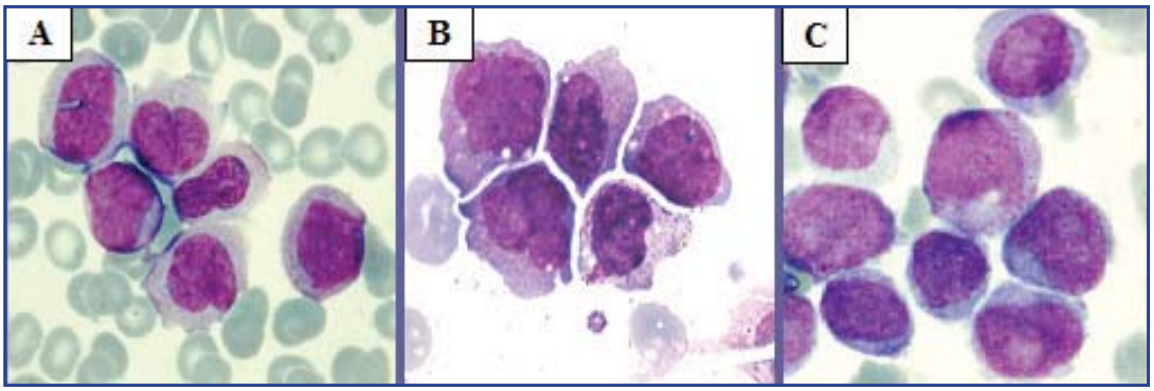

Fig. 1A. Bone marrow smears in patients № 1(A), № 2(B) and № 3(C). May-Grünwald Giemsa staining

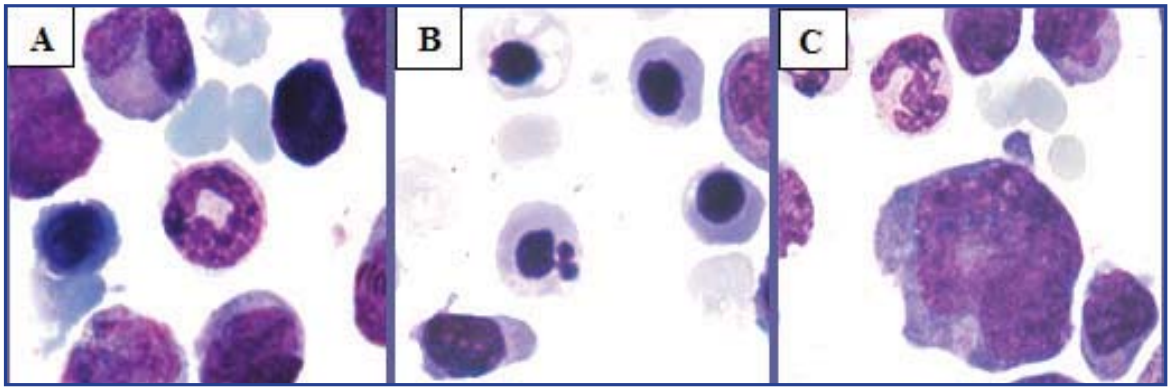

Fig. 2. Bone marrow cells with dysplastic changes (patient № 3): A) Granulocyte with ring-shaped nucleus; B) Erythroblasts with vacuolization of the cytoplasm and internuclear bridges; C) Small dysplastic megakaryocyte. May-Grünwald Giemsa staining

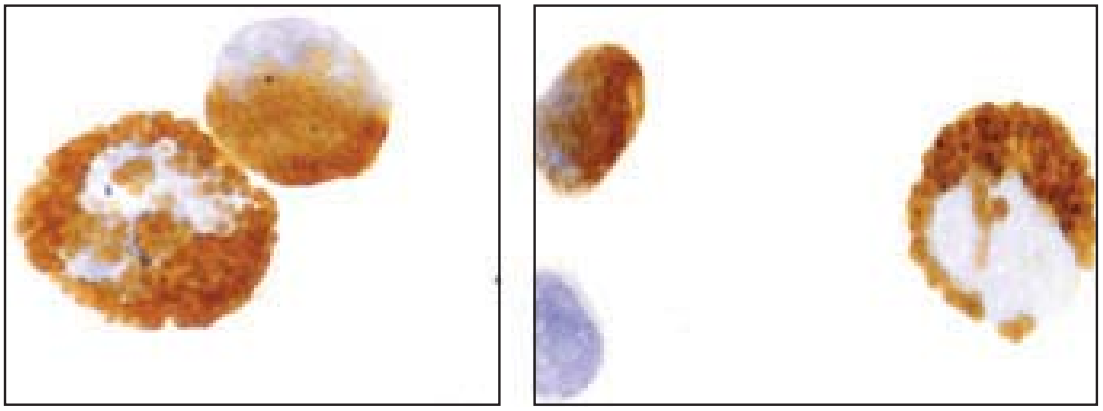

Fig. 3. Positive cytochemical reaction for myeloperoxidase in patients № 1 (A) and № 2 (B)

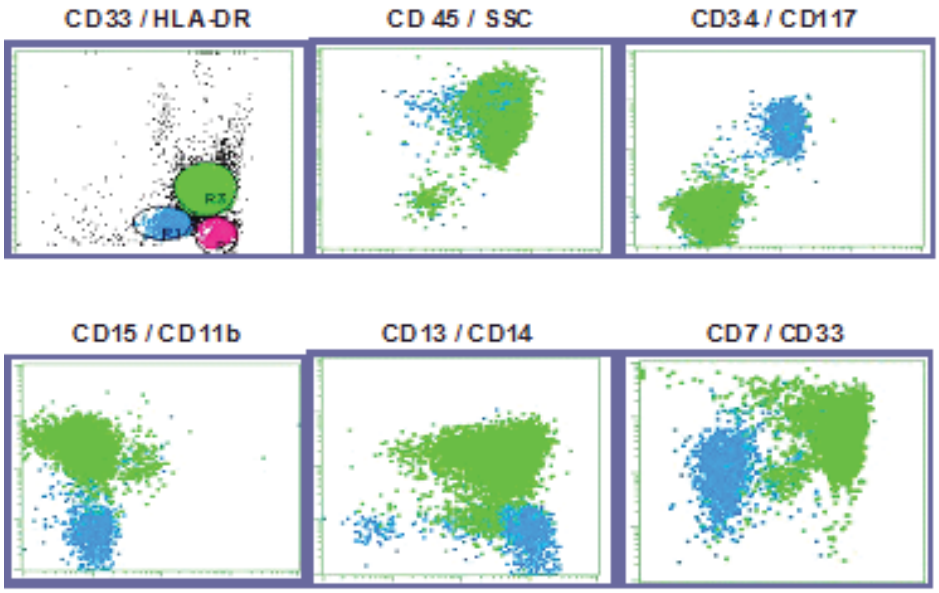

Fig. 4. Immunophenotyping of patient N1. Myeloid markers: CD13+, CD33+, CD34+, CD117+, CD7+, HLA-DR+, CD11b-, CD11c-, CD14-, CD15-, CD64-; Monocytoid markers: CD13+ CD33+, CD34-, CD117-, CD7+, HLA-DR+, CD11b+, CD11c+, CD14+, CD15-, CD64+

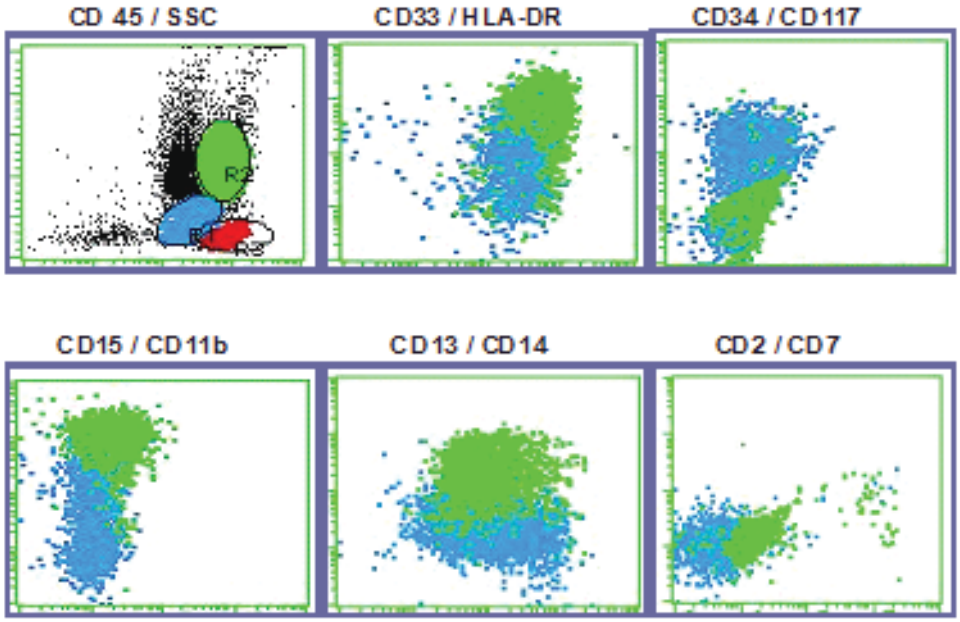

Fig. 5. Immunophenotyping of patient N2. Myeloid markers: CD13+, CD33+, CD117+, HLA-DR+, CD11b+/- CD11c+/-, CD34-, CD15-, CD14-, CD64-, CD2-, CD7-; Monocytoid markers:CD13+, CD33+, CD117-, HLA-DR+, CD11b+, CD11c+, CD34-, CD15-, CD14+, CD64+, CD2-, CD7-

Cytogenetic characteristics: Deletion of the long arm of chromosome 9 was found as the sole abnormality in all of the cases, with different interstitial regions

being involved. Karyotypic analysis included a specification of the breakpoints defining the deleted region in 3 patients. Karyotyping indicated two regions of deletion – 9q21–22 and 9q13-34. This is schematically illustrated in Fig. 6. Cytogenetic findings were presented in Fig. 7 and Fig. 8.

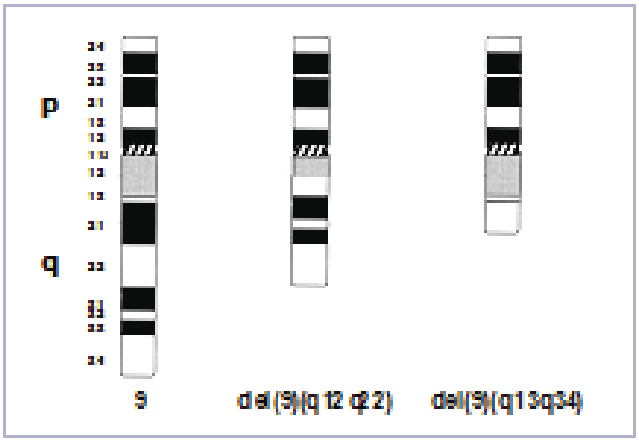

Fig. 6. Schematic representation of the deleted segments of 9q in del(9)(q12;q34) and del(9)(q13;q34)

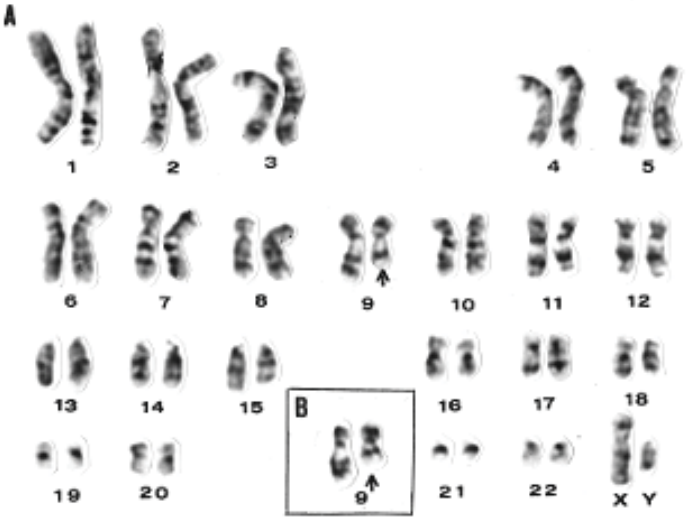

Fig. 7. A. Karyotype of patient № 1 with del(9)(q12q22); B. Partial karyotype del(9)(q12q22)

Fig. 8. Partial karyotype del(9)(q13q34) in patient № 2

DISSCUSION

Analysis of the cytogenetic abnormalities in AML patients is important for correct diagnosis and prediction of the disease prognosis [3, 7].

Deletion of a portion of the long arm of chromosome 9, del(9q), is a recurring abnormality in malignant myeloid diseases reported in approximately 2% of all AML cases [1]. After numerical abnormalities, del(9q) is the single most common associated structural chromosomal abnormality seen together with t(8;21) in AML, indicating that loss of function of a gene or genes on chromosome 9q may be one of the most important cooperating genes in t(8;21) AML [2, 12].

In this study we have analysed the clinical, immunological and cytogenetic characteristics of patients with del(9q) as the sole karyotypic anomaly. We found the association of del(9q) with characteristic phenotypic abnormalities: very immature blasts with or without cytoplasmic granulations, dysplastic changes in granulocyte lineage, giant myeloperoxidase positive cytoplasmic granulations (pseudo-Chediak-Higashi type) and CD7(+) and CD34 (+)/CD34(-) antigen expressions. There was also an association with dysplastic changes in erythroid, monocytoid and megakaryocytic lineages. It is likely that del(9q) is a clonal event in the pre-leukemic disease stage, probably in unrecognized MDS [8]. Deletion of tumor-suppressor genes in 9q chromosome regions may be related to leukemogenesis in AML.

Based on our study, we conclude that del(9q) cytogenetic abnormalities were associated with two specific morphological subtypes of AMLs: 1. AML/del(9q) with hypogranular CD34(+) and CD7(+) immature blasts and 2. AML/del(9q) with more mature granular CD34(-) and CD(-) blasts.

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