Clonal evolution of monosomy 7 in acquired severe aplastic anemia: Two cases treated with allogeneic hematopoietic stem cell transplantation

Edinilmiş ağır aplastik anemide monozomi 7 ile klonal evrim:
Allojeneik hematopoietik kök hücre transplantasyonu yapılan iki olgu

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Abstract

Aplastic anemia (AA) may evolve into clonal diseases like myelodysplastic syndrome (MDS) and acute myeloblastic leukemia (AML). Monosomy 7 is a poor prognostic chromosomal abnormality commonly associated with therapy related MDS and secondary AML. It has also been associated with leukomogenic transformation in AA. We present here two adult male patients with acquired severe AA. Both patients had received immunosuppressive therapy (IST) as first line treatment and had monosomy 7 positive clone at transformation to MDS with refractory anemia and excess of blast (RAEB-II) and AML, respectively. Both patients have undergone allogeneic hematopoietic stem cell (HSC) transplantation from their HLA identical donors (unrelated and sibling). *(Turk J Hematol 2008; 25: 94-7)*

Anahtar kelimeler: Aplastic anemia, clonal disease, monosomy 7

Özet

Aplastik anemi (AA), miyelodisplastik sendrom (MDS) ve akut miyeloblastik lösemi (AML) gibi klonal hastalıklara dönüşüm gösterebilmektedir. Monozomi 7, tedaviyle ilişkili MDS ve sekonder AML ile birlikte sıkça gözlenen kötü prognostik bir kromozomal anomalidir. Bunun yanısıra AA'da lökomojenik transformasyonla da ilişkilendirilmiştir. Burada ilk sıra tedavi olarak immünsupresif tedavi almış ve sırasıyla blast artışıyla giden refrakter anemi (MDS-RAEB-II) ve AML'ye transforme olmuş iki ağır AA'lı olgu bildirmekteyiz. Hastalarda transformasyon sırasında monozomi 7 klonunun pozitif olduğu saptanmıştır. Her iki hastaya da HLA uyumlu vericilerinden (akraba dışı ve kardeş) allojeneik hematopoietik kök hücre transplantasyonu yapılmıştır. *(Turk J Hematol 2008; 25: 94-7)*

Anahtar kelimeler: Aplastik anemi, klonal hastalık, monozomi 7

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Introduction

Aplastic anemia may evolve into clonal diseases like MDS, AML and paroxysmal nocturnal hemoglobinuria. The actuarial risk of MDS and acute leukemia in AA patients is 10-20% at 10 years [1,2]. Autoimmune destruction of the hematopoietic stem cells plays a major role in the pathogenesis and results in hypocellular bone marrow (BM) and peripheral blood pancytopenia. Thus, the current treatment for severe AA is IST and/or HSC transplantation considering the age of the patient. It has been pointed that the main causes of death in long term survivals of AA patients after IST are evolving clonal diseases like AML and MDS [3].

Cytogenetic abnormalities in AA are infrequent and their relevance in the disease course is not clear. Monosomy 7 is a karyotypic abnormality commonly associated with alkylating agent related MDS and AML and has a very poor prognosis. It has also been associated with leukomogenic transformation in AA [4,5]. However, the factors which affect the leukomogenic transformation of AA are not well defined. It is not clear whether this is a natural behavior of the disease which emerges during long term survival after IST or is simply therapy related. Evidence suggests that genetically unstable clones are present in AA patients who later develop MDS [6]. It has been suggested that an injury which generates an autoimmune reaction against HSC or progenitors in the BM can also induce a genetic mutation in the HSC, which subsequently results in clonal expansion [7].

Two adult patients with acquired AA were discovered to have clonal evolution to MDS and AML, respectively. Patients were treated with allogeneic HSC transplantation. Both patients had monosomy 7 clone at the time of evolution. Only cytogenetic analysis was available at the time of initial diagnosis of AA. Both cytogenetic and fluorescence in situ hybridization (FISH) analysis of the BM were performed at the time of evolution.

Case 1

A 20 year old male patient was diagnosed as very severe aplastic anemia in the year 2001. His CBC at diagnosis revealed WBC 0.6 x10^9/l, ANC 0.2 x10^9/l, Hb 8.5 gr/dl, platelet count 4x10^9/l. Bone marrow biopsy was consistent with AA revealing <5% cellularity and without any displastic signs (Figure 1). Cytogenetic analysis was not successful due to insufficient generation of metaphases. Since the patient did not have an HLA full match sibling donor, he received a course of IST. Failing to respond to the first course of IST with antilymphocyte globulin and cyclosporin A (CsA) at six months, he received a second course of IST (Antithymocyte globulin and CsA extended to 12 months) with a partial response. Three years after diagnosis, he was in complete remission (WBC 4.5 x10^9/l, Hb 11.2gr/dl, platelets 392x10^9/l). However, after one year, he presented with pancytopenia (WBC 2.6x10^9/l, ANC 0.9x10^9/l, Hb 7.4 gr/dl, platelets 108x10^9/l) and BM biopsy was consistent with MDS-RAEB-II (Figure 2). Conventional cytogenetics revealed karyotype 45,XY,-7 [7] with hypodiploidy in all 17 metaphases counted and clonal monosomy 7. FISH analysis demonstrated 37.5% del 7 in counted 500 interphases (Figure 3). His BM recovered with minimal residual disease after the remission induction chemotherapy with cytarabine and daunorubicin. The disease progressed with the expansion of the monosomy 7 clone to 88% in the following months. His follow up BM biopsy revealed hypercellular BM with early forms of erythro-

id and myeloid series, continuing dysplasia. He received two courses of 5-azacytidine without an apparent response. Approximately nine months after transformation to MDS, the patient received allogeneic peripheral stem cell transplantation from an
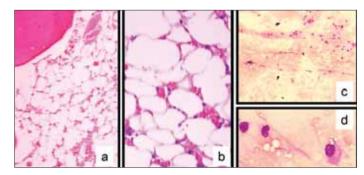

Figure 1. The first bone marrow biopsy of case 1 (2001) diagnosed as aplastic anemia. It was hypocellular without any hematopoietic elements (a,b). The aspiration smear revealed lymphocytes and plasma cells (c,d).
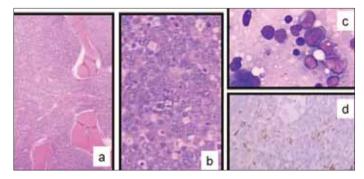

Figure 2. The bone marrow biopsy of case 1 in 2005 was diagnosed as RAEB-II. The biopsy was hypercellular (a) with increased immature erythroid precursors (b,c) and CD34 positive blasts (c,d).
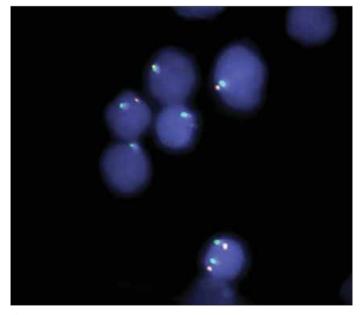

Figure 3. FISH analysis of monosomy 7. One probe (orange) was used to evaluate the band7q31 (LSI D7S486) region of chromosome 7 and the other probe (green) was used to evaluate the 7p11.1-q11.1 locus D7ZI region at the centromere. In normal interphase nucleus, two signals from each probe are obtained. However, in presence of monosomy 7, only one signal from each probe is obtained.

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HLA matched, sex mismatched, unrelated donor. At day +100 the patient was in complete remission with full donor chimerism and without any sign of acute GVHD. Moreover, monosomy 7 clone could not be deteced on FISH analysis in bone marrow samples. However, he died of a severe chronic gastrointestinal tract GVHD on the +145th day.

Case 2

A 34 year old male patient was diagnosed as severe AA 3 years ago. He received one course of IST (Horse antithymocyte globulin and CsA). Cyclosporine A was continued for up to one year. He was transfusion independent for a year when last year he presented with an elevated leukocyte count (WBC 34.9x10⁹/l, Hb 10.⁹gr/dl and platelets 19 x10⁹/l). His peripheral smear revealed blasts and BM aspirate and biopsy were consistent with AML. Conventional cytogenetics revealed 45, XY, -7 [18]. After remission induction therapy (cytarabine, idarubicin) his BM revealed aplasia with minimal dysplastic changes. Both conventional cytogenetics and FISH analysis could not reveal monosomy 7. He received allogeneic BM transplantation from his HLA identical sister. He experienced Grade II skin and Grade III acute hepatic GVHD. At day +100 he was in complete remission with 100% donor chimerism. No sign of monosomy 7 clone was detectable in bone marrow interphase FISH analysis. He is still alive and in complete remission 18 months after transplantation.

Discussion

The prognostic factors which predict leukomogenic transformation of severe AA are not well defined. Chromosomal abnormalities which are present at diagnosis or emerge during the course of the disease may have clinical impact. Monosomy 7 is associated with leukomogenic transformation and encapacitates poor prognosis. There are many publications confirming that the development of MDS and acute leukemia is increased after IST [8,9]. However, the evidence of clonal diseases before the introduction of IST (esp. ATG) to the standart treatment of AA suggests that IST is not the only contributing factor for clonal evolution [10,11]. Hematopoietic stem cell transplantation is recommended as first line therapy for patients younger than 40 years and with an available matched sibling HSC donor [12]. Both cases reported here were younger than 40 but they had to receive IST as first line treatment due to unavailability of a suitable donor at diagnosis. Tichelli et al treated 103 AA patients with ALG and reported 20 patients who developed MDS and/or PNH. Three patients demonstrated monosomy 7 at the time of clonal evolution. On the contrary, 34 patients who were treated with BM transplantation did not show any clonal evolution [8]. Socie et al reported 34 patients with MDS or acute leukemia out of 860 patients treated with IST in contrast to only 2 patients out of 748 patients treated with BM transplantation in EBMT Study [9]. Kearns et al reported 13 cytogenetic abnormalities out of 47 AA patients. Monosomy 7 was the most common. None of the monosomy 7 patients subsequently developed AML but they had poor prognosis [13]. On the contrary, Piaggio et al could not demonstrate any clinical impact of chromosomal abnormalities emerged during the course of IST on survival or transfusion independence [14].

There are competing reports for the contribution of prolonged administration of rhG-CSF as a factor for clonal evolution in AA.

Most of the studies concluded that long term (>1 year) use of rhG-CSF and no response to IST at 6 months are significant risk factors for the development of MDS [15-18]. Our patients reported here had either poor or incomplete response to IST at six months but none of them received long term rhG-CSF. Ohara et al reported that 11 out of 50 children with AA who were treated with CsA and rhG-CSF developed MDS. Ten of these children had monosomy 7 at diagnosis of MDS [16]. Kaito et al reported 5 cases of MDS out of 72 adult AA patients. Four patients had received IST and presented with monosomy 7 within 3 years. All patients had received G-CSF for more than a year [17]. On the contrary, Locasciulli et al treated 144 adult severe AA patients with IST with or without rhG-CSF. The risk of MDS/AML was similar in both groups. They concluded that large doses of rhG-CSF administration over a long time in addition to IST does not increase the risk of clonal diseas in AA [19]. Gluckman et al treated 102 patients with IST with or without rhG-CSF. In the first 12 weeks of treatment and after a median follow up of 5 years no difference was observed in terms of survival, hematologic response or secondary leukemia [20]. It has been very recently suggested by Sloand et al that pharmacologic doses of G-CSF increase the proportion of preexisting monosomy 7 cells. The abnormal response of monosomy 7 cells to G-CSF was explained by the expansion of undifferentiated monosomy 7 clones expressing the class IV G-CSF receptor, which is defective in signaling cell maturation but can induce proliferation [21].

Whether the monosomy 7 clone has been present from the begining at diagnosis or emerged later during the course of the disease is a matter of speculation in our reported patients. FISH analysis was unavailable for both patients at the time of diagnosis. Conventional cytogenetics could not reveal any metaphases at the end of 24 hour culture. It should be noted that the use of conventional cytogenetics in bone marrow failure syndromes may be limited because of the absence of dividing cells to enable analysis of metaphase chromosomes. FISH can detect aneuploidy in non-dividing interphase cells so it can reveal positive results in AA patients who had otherwise normal karyotype with conventional cytogenetics [13]. Some of the chromosomal abnormalities may be present at the diagnosis and may have been missed in routine cytogenetic studies due to lack of metaphases. FISH should be added to the routine genetic work up both at initial diagnosis and during the follow up in AA.

Although one hypothesis points that the leukemic clones in AA escape from anti-tumor immunity because of IST and their proliferation is further accelerated by rhG-CSF, the distinct biological pathways linking monosomy 7 to malignant transformation in AA have not been defined so far [17]. Monosomy 7 in AA should be regarded as a preleukemic clonal state which can evolve into MDS or AML and when transformed, it has poor response to chemotherapy and early HSC transplantation should be considered during the disease course.

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