Monosomy 7 in Patients with Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria with Evolution into Acute Myeloid Leukemia

Chirayu Udomsakdi Auewarakul MD, PhD*, Chintana Tocharoentanaphol PhD**, Wanchai Wanachiwanawin MD*, Surapol Issaragrisil MD*

** Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University*
*** DNA Center, Bangkok*

We report two cases of Thai patients with aplastic anemia/paroxysmal nocturnal hemoglobinuria (AA/PNH) who subsequently developed acute myeloid leukemia (AML) at their terminal phase. Monosomy 7 was demonstrated upon karyotypic analysis of bone marrow in both cases at the time leukemia developed. The first patient was a 25-year-old man diagnosed with AA at age 14, recovered from AA at age 15, developed PNH at age 21 and turned into AML at age 25. The second patient was a 27-year-old man diagnosed with PNH at age 22, developed severe AA at age 25 and turned into AML at age 27. This latter patient received anti-lymphocyte globulin when he developed severe AA but did not respond well whereas the first patient fully recovered from AA with anabolic hormone treatment. Time to diagnosis of AML in the patient who received immunosuppressive therapy was strikingly shorter than that who received conventional androgen therapy (2 years vs 11 years after AA, respectively). The presence of monosomy 7 in leukemic cells of both patients emphasizes its central role in the development of AML from AA/PNH. However, other factors such as choice of AA/PNH therapy and patient's response may modulate the time to emergence of monosomy 7-carrying AML clone and frank leukemia. Further studies into the biologic and genetic mechanisms involved in the development of leukemic clone arising from AA/PNH should be explored.

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Aplastic anemia (AA) is a disease of hematopoietic stem cells presenting with peripheral blood pancytopenia as a result of acquired bone marrow failure[1]. It is a more common disease in Asia than in the West with the annual incidence in Thailand of 3-5 per million in contrast to 1-2 per million in Europe[2,3]. Various clonal stem cell disorders have been reported to be associated with AA, including paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)[4-6]. Clinical PNH can occur before or after the diagnosis of AA whereas MDS most frequently arises after the treatment of AA.

The association of AA-PNH and acute leukemia was first speculated in Thailand by Wasi et al in 1970 and confirmed by later reports from many countries[7]. The incidence of clonal stem cell disorders appeared to increase after the introduction of immunosuppressive therapy in Western countries with the incidence of MDS and acute leukemia following AA estimated to be about 5%[8,9].

The true incidence of acute leukemia after AA in Thailand is not known but appears to be infrequent. In the present study, the authors report two cases of AA/PNH patients who developed acute leukemia during their terminal course. Karyotypic analysis of leukemic cells was performed and revealed monosomy 7 as the frequent karyotype. Clinical characteristics and outcomes of the patients are presented. Cases of monosomy 7-carrying AML cells arising from AA patients in the literature were also discussed.

Correspondence to : Auewarakul CU, Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-7643, Fax: 0-2412-9783, E-mail: sicaw@mahidol.ac.th

Case Report

The first patient (HN 40-055784) was a 14-year-old student who was diagnosed with severe

aplastic anemia in December 1990 when he presented at Siriraj Hospital with pallor and low-grade fever. His complete blood counts (CBC) were as shown in Table 1. Stem cell transplantation was considered but HLA types of his only sister was non-identical. He was given oxymethalone 50 mg orally three times a day as well as transfusion support. He did not receive any immunosuppressive drugs and gradually improved with conventional anabolic hormone therapy. His CBC became normalized one year after the diagnosis of aplastic anemia. He was thus taken off anabolic hormone in June 1991 and did well until 1997 when he again became anemic with hemoglobin (Hb) level of 7.0 g/dL, a normal white blood cell (WBC) count of $4.8x10^9$/L and a platelet count of $176x10^9$/L. Ham's test and urine hemosiderin was positive and he was diagnosed as PNH. He was then treated with prednisolone 60 mg orally every other day with daily ferrous sulfate and folic acid supplementation. The patient's anemia slightly improved and he was lost to follow-up for another 4 years. In March 2001, the patient returned with gum bleeding, petechiae, fatigue, and high-grade fever. His CBC at this time revealed an elevated WBC of $19.6x10^9$/L, platelet count of $10x10^9$/L and Hb of 3.6 g/dL. Numerous monocytes and blasts were seen upon review of peripheral blood smear. Bone marrow studies confirmed the diagnosis of AML and flow cytometry revealed blasts with monocytic markers. Monosomy 7 and 21q+ was demonstrated by Q-banding method. He was treated with cytarabine 200 mg/day for 7 days and doxorubicin 60 mg/day for 3 days. He persistently had a high fever despite broad-spectrum intravenous antibiotics and succumbed to death at age 25 due to massive upper gastrointestinal (GI) bleeding in April 2001.

The second patient (HN 42-001897) was a 22-year-old man who presented with anemia and dark urine and was diagnosed as PNH in 1994 at another university hospital in Thailand. He was treated with prednisolone and transfusion support with a slight clinical improvement of his anemia. In February 1998, he presented to Siriraj Hospital and was found to have marked pancytopenia as shown in Table 1. Ham's test and urine hemosiderin were positive. Bone marrow biopsy revealed marked hypocellularity consistent with severe aplastic anemia. In August 1998, the patient was treated with horse anti-lymphocyte globulin (ALG) for 5 days. He did not respond and continued to have pancytopenia and hemoglobinuria. His course was further complicated by pulmonary tuberculosis requiring multiple anti-tuberculosis drugs in December 1999. In June 2000, the patient complained of fever with chills and abdominal discomfort. He was admitted to the hospital and found to have anemia, fever with an enlarged liver of 4 centimeters below the right costal margin and an enlarged spleen of 3 centimeters below the left costal margin. Blasts with numerous nucleated red blood cells were seen upon review of his peripheral blood smear. Bone marrow morphological and cytochemical studies were compatible with erythroleukemia (M6 by FAB classification). Chromosome study of his bone marrow cells showed monosomy 7 (Fig. 1). He received cytarabine 300 mg/day for 7 days and doxorubicin 50 mg/day for 3 days. The patient continued to have high fevers and was later found to have E.coli septicemia for which he was treated. He was also continued on anti-tuberculosis drugs. The patient eventually developed frank lower GI bleeding and platelet

Table 1. Clinical and hematologic parameters of Patients 1 and 2

	Age	Hemoglobinuria	Hepatomegaly/ Splenomegaly	Anemia	WBC ($x10^9$/L)	Hb (g/dL)	Plt ($x10^9$/L)	Ham's test
Patient 1								
At diagnosis of AA	14	no	no/no	yes	2.8	3.3	15	ND
After hormone treatment	15	no	no/no	no	7.1	14.9	200	ND
At diagnosis of PNH	21	yes	no/no	yes	4.8	7.0	176	positive
At diagnosis of AML	25	no	yes/no	yes	19.6	3.6	10	ND
Patient 2								
At diagnosis of PNH	22	yes	no/no	yes	NA[a]	NA[a]	NA[a]	positive
At diagnosis of AA	25	yes	no/no	yes	1.9	3.8	2	positive
After ALG treatment	26	yes	no/no	yes	2.2	4.4	28	positive
At diagnosis of AML	27	no	yes/yes	yes	13.3	3.6	6	ND

[a] Data from a referring hospital not available

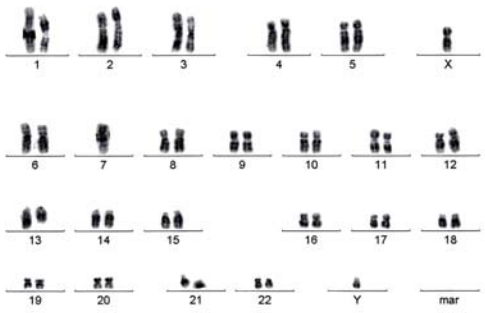

Fig. 1 A representative karyotypic analysis of bone marrow at the time of AML development showing monosomy 7 (Patient 2)

refractoriness and died at the end of June 2000 at age 27.

Discussion

Secondary AML is known to occur after a number of chronic bone marrow disorders and exposure to DNA-damaging chemotherapeutic agents[10]. The evolution into AML following MDS and chronic myeloid leukemia (CML) is an expected terminal event. In the past, secondary AML was uncommon in AA or PNH patients who were usually considered to have a more benign clinical course. With the introduction of immunosuppressive drugs including ATG, cyclosporineA, and cyclophosphamide, the outcome of severe AA patients in the western countries has much improved over the past 20 years[11,12]. The increased long-term survival of severe AA patients in the western countries, however, has revealed emerging problems and increased later complications such as MDS and AML[8,9, 12]. In contrast to the West, most Thai AA patients continued to receive conventional therapy including anabolic hormones and steroids and their major cause of death came from pancytopenia and its related complications (unpublished observation).

Despite the difficulties associated with karyotypic analysis of bone marrow containing sparse hematopoietic cells in the setting of severe AA, various chromosome abnormalities have been reported including trisomy 6, trisomy 8, monosomy 7, and trisomy 21[13-17]. In a recent European analysis, chromosome abnormalities were found in 4 of 170 AA patients at diagnosis[8]. Among 69 Italian patients, trisomy 8 was most frequent, present in 8 of 18 cases, followed by monosomy 7 in 2 of 18 patients[13]. A recent meta-analysis, however, showed trisomy 6 as the most frequent chromosome abnormality in AA[15]. Trisomy 6 seems to be more common at diagnosis while monosomy 7 is more common after therapy.

Abnormal karyotypes have been variably encountered during the later course of AA and were associated with different clinical outcomes. Socie et al studied the incidence of malignant tumors in a large number of AA patients and found 5 cases of AML (M4 by FAB Classification) and 6 cases of MDS, all with monosomy 7[6]. All 5 of 5 monosomy 7-positive AML cases died while 4 of 6 MDS patients with refractory anemia were still alive. In Japanese children with AML arising from AA, monosomy 7 was found in 6 of 113 cases[16]. Similar frequency was also reported by an American series[18].

Maciejewski et al recently reported the results of karyotypic analysis of 30 American AA patients; 3 of which had monosomy 7 at the time of development of AML and 6 cases had monosomy 7 at the time of development of MDS[19]. Monosomy 7 accounted for 40% of all cases of AA with clonal evolution and was seen most often in refractory patients who had failed to respond to therapy. Other abnormalities include trisomy 8, structural and numerical abnormalities of chromosome 13, deletion of Y chromosome and complex cytogenetic abnormalities. Trisomy 8 developed in the patients with good responses who often required chronic immunosuppression with cyclosporineA and had excellent outcome. Most deaths related to leukemic transformation occurred in patients with chromosome 7 abnormalities or complex cytogenetic alterations or both while all patients with trisomy 8 remained alive and did not progress to leukemia over a mean observation interval of 44 months.

The explanation for the development of a leukemic clone from AA is presently uncertain[20,21]. One concept is that AA is a premalignant disease and the increased survival time displays its malignant natural history while the other concept is that the secondary malignant disease is causally related to the therapy itself including treatments with ATG, cyclosporinA, cyclophosphamide or G-CSF. Our first case epitomizes the concept of premalignant nature of AA while the second case may represent the effect of therapy imposed on the AA benign natural course. Monosomy 7 seems to play a central role in either situation. The presence of monosomy 7 in the presented Thai patients who succumbed to death with AML supports its unfavorable prognostic value

as pointed out by Maciejewski et al[19]. We do not know if the patients had monosomy 7 from the diagnosis of AA since karyotyping was not routinely performed at the initial diagnosis of AA at our hospital.

In conclusion, we demonstrate that monosomy 7 was present in both AML cases arising from AA/PNH and is associated with poor outcome. The choice of therapy and the patient's response seemed to modulate the natural history of the disease with the longer time to the diagnosis of AML in the patient who responded to conventional therapy. In Thailand, the true incidence of AML after AA or PNH is not known but may increase in the future with more utilization of immunosuppressive drugs in Thai patients. Further studies into the biologic and genetic mechanisms involved in the development of leukemic clone arising from AA/PNH should be explored.

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ความผิดปกติของโครโมโซมคู่ที่ 7 ในเซลล์ของผู้ป่วยโรคไขกระดูกฝ่อสองรายที่กลายเป็นมะเร็งเม็ดเลือดขาวชนิดเฉียบพลัน

จิรายุ เอื้อวรากุล, จินตนา โตเจริญธนผล, วันชัย วนะชิวนาวิน, สุรพล อิสรไกรศีล

มะเร็งเม็ดเลือดขาวชนิดเฉียบพลันพบได้ไม่บ่อยในผู้ป่วยไทยที่เป็นโรคไขกระดูกฝ่อ หรือ ผู้ป่วยที่เป็นโรคโลหิตจางที่มีปัสสาวะดำจากเม็ดเลือดแดงแตก (หรือย่อว่า โรคพีเอ็นเอ็ช) ผู้นิพนธ์รายงานการตรวจพบความผิดปกติทางโครโมโซมในเซลล์ไขกระดูกของผู้ป่วยไทย สองรายที่เดิมเป็นโรคไขกระดูกฝ่อ และต่อมากลายเป็นมะเร็งเม็ดเลือดขาวมัยอีลอยด์ชนิดเฉียบพลัน พบว่าในทั้งสองรายมีความผิดปกติของโครโมโซมคู่ที่ 7 โดยมีการขาดหายของชิ้นส่วนของโครโมโซมคู่ที่ 7 ในทั้งสองราย ผู้ป่วยรายที่หนึ่งเป็นผู้ป่วยชายอายุ 14 ปีเมื่อแรกวินิจฉัยว่าเป็นโรคไขกระดูกฝ่อ 7 ปีต่อมากลายเป็นโรคพีเอ็นเอ็ช และในที่สุดกลายเป็นมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันเมื่ออายุ 25 ปี ผู้ป่วยรายที่สองมีอายุ 22 ปีเมื่อได้รับการวินิจฉัยว่าเป็นโรคพีเอ็นเอ็ช ต่อมากลายเป็นโรคไขกระดูกฝ่อเมื่ออายุ 25 ปี และในที่สุดกลายเป็นมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันเมื่ออายุ 27 ปี ผู้ป่วยรายที่หนึ่งไม่ได้ยากดภูมิคุ้มกัน และเกิดมะเร็งในระยะเวลา 11 ปีนับจากที่เริ่มป่วยเป็นโรคไขกระดูกฝ่อ รายที่สองได้ยากดภูมิคุ้มกัน และเกิดมะเร็งขึ้นในระยะเวลา 2 ปีหลังจากได้รับการวินิจฉัยว่าเป็นโรคไขกระดูกฝ่อ ทั้งสองรายเสียชีวิตในเวลาไม่นานหลังกลายเป็นมะเร็ง โดยสรุป ความผิดปกติของโครโมโซมคู่ที่ 7 พบได้ในโรคไขกระดูกฝ่อและโรคพีเอ็นเอ็ช และอาจบ่งบอกถึงพยากรณ์โรคที่ไม่ดี ความสำคัญของโครโมโซมคู่ที่ 7 ในกลไกการเกิดมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันในผู้ป่วยที่เป็นโรคไขกระดูกฝ่อ ยังไม่ทราบแน่ชัด แต่ควรมีการศึกษาเพิ่มเติม