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CASE REPORT

Reversal of Marrow Fibrosis in Agnogenic Myeloid Metaplasia by Allogeneic Peripheral Blood Stem Cell Transplantation

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SUMMARY **Agnogenic myeloid metaplasia (AMM) is a clonal hematopoietic stem cell disorder characterized by bone marrow fibrosis, extramedullary hemopoiesis, splenomegaly and a leukoerythroblastic blood picture. Current standard therapies using hydroxyurea, interferon, androgens or corticosteroids have not shown to prolong survival of patients with AMM. In this study, we performed a curative approach using an HLA-matched sibling as a donor for allogeneic peripheral blood stem cell transplantation (PBSCT) for a 45-year-old woman with AMM. Busulfan and cyclophosphamide were given as a conditioning regimen from day -7 to day -2 with cyclosporinA and methotrexate as post-transplant immunosuppressive therapy. Donor PBSCs were mobilized by G-CSF at 16 μg/kg/day for five days and transplantation was performed on March 2-3, 2000. The patient rapidly engrafted within 2 weeks after PBSC infusion without evidence of graft versus host disease. Her blood counts and bone marrow 2 years after transplantation were normal with full donor pattern by molecular analysis. In conclusion, marrow fibrosis can be reverted to normal by allogeneic PBSCT. Allogeneic PBSCT should thus be offered to AMM patients if an HLA-matched sibling is available. This report represents the first SCT for AMM in Thailand.**

Agnogenic myeloid metaplasia (AMM) is a chronic myeloproliferative disorder (MPD) characterized by progressive marrow fibrosis, extramedullary hematopoiesis, splenomegaly and a leukoerythroblastic blood picture.[1-2] The other members of chronic MPD include polycythemia vera (PV), essential thrombocythemia (ET) and chronic myeloid leukemia (CML), all of which can also be associated with myeloid metaplasia and marrow fibrosis, particularly at their terminal phase.[3] The primary pathogenetic mechanism of AMM is a malignant clonal hematopoietic stem cell disorder that leads to ineffective erythropoiesis, dysplastic megakaryocyte hyperplasia, and an increase in the ratio of immature granulocytes to total granulocytes.[4] The process of myelofibrosis is postulated to be the result of the stromal reaction of the bone marrow to the abnormal stem cells. Both megakaryocyte and monocytes in the marrow have been implicated as sources of cytokine production that stimulate fibroblast proliferation, collagen synthesis, angiogenesis and osteogenesis in AMM.[5]

AMM is a disease of adults and the elderly with the median age at diagnosis of 60 years.[6] Approximately 20% of patients are under the age of 55.[7] Current standard therapies using hydroxyurea, interferon, androgens or corticosteroids have not been shown to prolong survival of patients with AMM.[8-9] Splenic irradiation or splenectomy has often

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been utilized to relieve severe anemia and abdominal discomfort associated with massive splenomegaly.[10] Transfusion support to lessen severe anemia and heart failure is the mainstay of therapy for AMM patients at their terminal stage. Most patients die after a median survival of 3 years, giving AMM the worst prognosis among the chronic MPD. In recent years, bone marrow transplantation (BMT) has been used with increasing success to cure thousands of patients with hematologic disorders including acute leukemia, lymphoma, multiple myeloma and MPD. However, not as many patients with AMM were reported in the literature to receive BMT due to concerns that engraftment of stem cells in a fibrotic marrow might be unsuccessful.[11-15] In addition, the presence of marked splenomegaly in AMM patients also raises concerns about possible excessive sequestration of transplanted hemopoietic stem cells in the spleen and difficulties with transfusion support after transplantation.[16] Splenectomy performed prior to BMT was reported to fasten engraftment in some patients.[17]

AMM is not an uncommon MPD in Thailand, ranking second after CML. While we have cured many CML patients with BMT in Thailand, no case of AMM has been reported to receive such therapy. In this study, we report our first attempt to offer a curative therapy for a patient with AMM in Thailand using growth factor-stimulated allogeneic peripheral blood as a source of stem cells. The results of SCT in AMM patients in the literature are also discussed.

CASE REPORT

A 45-year-old Thai woman presented in August 1998 to Siriraj Hospital with weight loss, fatigue and abdominal discomfort. She had no prior medical illnesses and was taking no medications. Her physical examination revealed moderate anemia and an enlarged spleen that extended markedly down to the umbilicus. The rest of the examination was normal. Subsequently performed laboratory investigations are summarized in Table 1. A complete blood count (CBC) revealed a hemoglobin level of 8 g/dl, a white blood cell (WBC) count of 11.9 x 10^9/l and a platelet count of 30 x 10^9/l. The serum LDH level was 1,074 U/l (normal 225-450) and hepatic transaminases were normal. A leukoerythroblastic blood picture was evident upon review of her peripheral blood smears. Bone marrow aspiration revealed a dry tap as suspected. Core bone marrow biopsy was thus performed and the result confirmed the diagnosis of agnogenic myeloid metaplasia with myelofibrosis in this woman. A negative BCR-ABL gene also excluded the diagnosis of CML.[18] She initially received hydroxyurea with some minor improvement in symptoms and blood counts. In 1999, her splenomegaly increased markedly and her blood counts began to deteriorate. Hemoglobin decreased to 5.7 g/dl with a WBC count of 19.7 x 10^9/l and a platelet count of 19 x 10^9/l. The issue of BMT as a possible cure was discussed with the patient in August 1999 and it was decided to search for BMT donors. HLA typing of her family members was thus performed and yielded a perfectly matching sister. The patient subsequently underwent splenectomy to eliminate the potential problem of splenic sequestration of donor hemopoietic stem cells. She did well postoperatively without any complications. One month later she was admitted to Chulabhorn BMT Center for allogeneic peripheral blood stem cell transplantation (PBSCT).

Busulfan at 16 mg/kg and cyclophosphamide at 120 mg/kg were given as a conditioning regimen. Cyclosporin A and methotrexate were used as post-transplant immunosuppressive therapy. Donor PBSCs were mobilized by high dose treatment with granulocyte-colony-stimulating-factor (G-CSF) at 16 μg/kg/day for 5 days. The donor tolerated the subcutaneous G-CSF injections well without complications.[19] Allogeneic PBSCT was subsequently performed on March 2-3, 2000. The number of $CD34^+$ stem cells given was 1.7 x 10^7/kg. Rapid engraftment was observed in two weeks with an absolute neutrophil count (ANC) greater than 5 x 10^9/l on day 10 and a platelet count greater than 50 x 10^9/l by day 25 after transplantation. The patient did not produce an acute graft-versus-host disease or infections and was discharged on oral cyclosporin and bactrim. Bone marrow biopsy was performed at 3 months, 6 months and 2 years after transplantation. An initial bone marrow investigation at 3-6 months showed 50% marrow fibrosis and 50% normal hematopoietic elements while at 2 years a normal bone marrow with < 5% fibrosis was seen (Fig. 1). A CBC at 2 years showed a hemoglobin of 13.5 g/dl, a WBC count of 9.13 x 10^9/l and a platelet of 414 x 10^9/l. Molecular analysis of her bone marrow at 2 years post-transplant revealed a bone marrow pattern identical to the donor's. The patient was doing well and stopped taking cyclosporin after 1 year. She experienced no opportunistic infections

Table 1 Laboratory parameters before and after allogeneic PBSCT in a 45-year-old Thai woman

	At diagnosis	After PBSCT	
		At 6-month	At 2-year
Hemoglobin (g/dl)	8	10.3	13.5
WBC (x 10^9/l)	11.9	8.2	9.13
Plt (x 10^9/l)	30	470	414
Serum LDH	High	Slightly high	Normal
DNA pattern[a]	Recipient	Mixed chimerism	Donor

[a]Performed by Short-tandem-repeats (STR) molecular analysis

and chronic graft-*versus*-host disease except mild scleroderma-like lesions on both hands.

DISCUSSION

AMM is a clonal hemopoietic stem cell disorder with abnormal proliferation and accumulation of myeloid elements in the bone marrow. Extramedullary hematopoiesis or myeloid metaplasia arises as a result of bone marrow fibrosis. The biologic and molecular mechanisms responsible for clonal proliferation in AMM are presently unknown. Increased sensitivity of hematopoietic progenitor cells to growth factors is a well-known feature of AMM as well as other MPDs. Elevated expression and point mutation of stem cell factor receptor (c-kit) in circulating stem cells of AMM have also been reported and may contribute to the proliferative advantage of the abnormal stem cell clone.[20] A number of fibrogenic cytokines were found to be disregulated in AMM including transforming growth factor-beta and platelet-derived growth factor. In addition, thrombopoietin (TPO), a growth factor necessary for megakaryocyte development, has also been found to play a central role in AMM.[1,2] Overexpression of TPO or its receptor in murine models produces many of the clinical features of AMM.[21] Chronic exposure to TPO leads to thrombocytosis, megakaryocyte hyperplasia in the spleen and bones, extramedullary hematopoiesis, myelofibrosis, and osteosclerosis.[22]

Although the concept of a reactive fibrotic process to abnormal stem cells has been accepted for many years, only a few studies were reported prior to 1999 with regards to the use of allogeneic BMT to treat AMM patients. The mainstay of treatment for AMM, including hydroxyurea, steroids, androgens, alpha-interferon may have benefits in terms of palliative care but does not translate into improved overall survival. Management for the majority of patients is thus directed towards the alleviation of symptoms and improvement in quality of life. Abnormal stem cells are by no means completely eliminated by conventional therapies. Hematopoietic SCT using allogeneic cells should thus be the only potential of cure in this group of patients. The largest series of allogeneic BMT in AMM patients to date was reported by Guardiola *et al.*[23] in May 1999. In this study, 55 patients from 27 centers in Europe and North America were transplanted. Most patients were young with the median age of 42 years, similar to our patient and the median time to transplant was 21 months, slightly longer than our patient's time to transplant of 19 months. The 5-year survival in their study was 47% and treatment failure developed in 13 of 55 patients.

It was striking to note that SCT using peripheral blood was even less frequently performed than BMT for AMM patients despite the fact that PBSCT has been shown to cure many hematologic disorders and has now become a standard procedure in many transplant centers worldwide. Even in the largest retrospective study reported by Guardiola *et al.*,[23] only 6 patients received PBSCT while the majority of patients received BMT. It was not investigated whether the source of the stem cells had any differential impact on the outcome of AMM patients. Following this largest study, only 3 AMM patients from Seattle were reported to receive allogeneic PBSCT[17] in 2001 and 4 AMM patients from Chicago received reduced-intensity conditioning regimen followed by allogeneic PBSCT in 2002.[24] All patients in the latter study engrafted well and had stable full-donor chimerism. This result is consistent with our present report that shows PBSC could engraft well even in extensively myelofibrotic marrow. Overall, fewer than 20 AMM patients had reportedly undergone allogeneic PBSCT worldwide.

The role of splenectomy in AMM is uncertain. In 1998, Barosi *et al.*[25] reported of a large series of 549 AMM patients, 87 of whom were

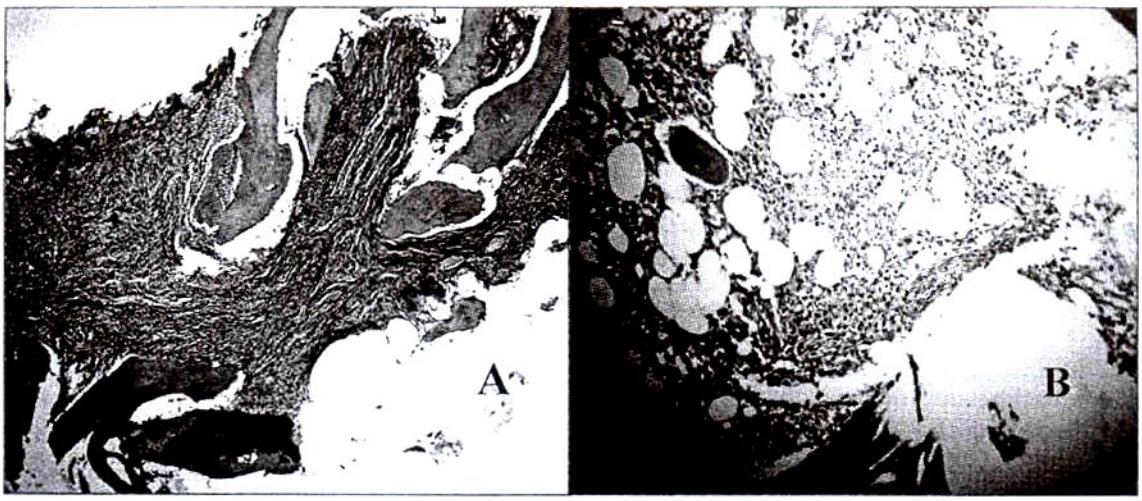

Fig. 1 Bone marrow biopsy specimens before (A) and 2 years (B) after allogeneic SCT. Extensive marrow fibrosis is seen in A and normal marrow hematopoietic elements in B.

splenectomized. It was found that patients who underwent splenectomy developed more blast transformation than patients who were not splenectomized (26% *vs* 12%). Tefferi *et al.*[26] subsequently analysed the outcome of 223 AMM patients who underwent splenectomy. Although the rate of blast transformation was high (16%), the overall survival after splenectomy was not compromised. Thus, they suggested that an appropriate surgical intervention should not be waived for fear of increased risk of leukemia. The role of splenectomy in conjuction with stem cell transplantation is even less certain due to the small number of patients studied. Li *et al.*[17] analysed the outcome after allogeneic transplants in 26 AMM patients, 11 of whom had splenectomy prior to transplant. The overall survival was not different between the two groups (73%, splenectomized *vs* 64%, nonsplenectomized, p = ns) but patients with splenectomy had faster granulocyte recovery. This was in agreement with the Guardiola Study that showed that splenectomy, the absence of grade-3 myelofibrosis, and a high number of nucleated cells infused were factors associated with rapid engraftment. In our study, we elected to perform splenectomy prior to PBSCT due to the concern of splenic sequestration of donor stem cells in the recipient's huge spleen.

Our study showed that SCT using G-CSF-stimulated peripheral donor blood is feasible for AMM patients and the process of marrow fibrosis can be reverted to normal with this treatment. It is of interest to see that marrow fibrosis may take time to disappear as demonstrated in our patient, where at least half of the biopsied marrow at 3-6 months still showed fibrotic areas. A complete reversal of myelofibrosis may take as much time as 1-2 years after SCT.[27] Despite significant relapses demonstrated in 10 of 55 AMM patients in the Guardiola Study, reinduction into remission was also possible. Donor lymphocyte infusions (DLI) have been utilized in some patients who relapsed after SCT to create the so-called graft-versus-myelofibrosis effect.[28-29] Myeloablation followed by autologous SCT was also recently reported and showed clinical responses in some AMM patients.[30]

In summary, we report the first use of allogeneic PBSCT in a Thai AMM patient with the disease progressed on conventional therapy. SCT appears to be the only ef-

fective treatment to cure AMM patients. The 5-year disease-free survival rate after SCT from the Seattle series was around 50% although patients under the age of 45 tended to have a better outcome.[31] Decision regarding the appropriate time to perform transplantation is still not known. The development of strategies to identify distinct prognostic groups of AMM patients may help physicians to select high-risk patients for immediate transplantation.[32] A prospective multicenter trial should be established to determine the role of allogeneic PBSCT as well as the variables that would affect the transplant outcome in AMM patients.

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